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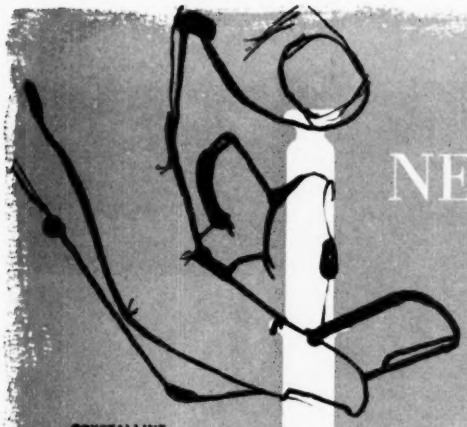

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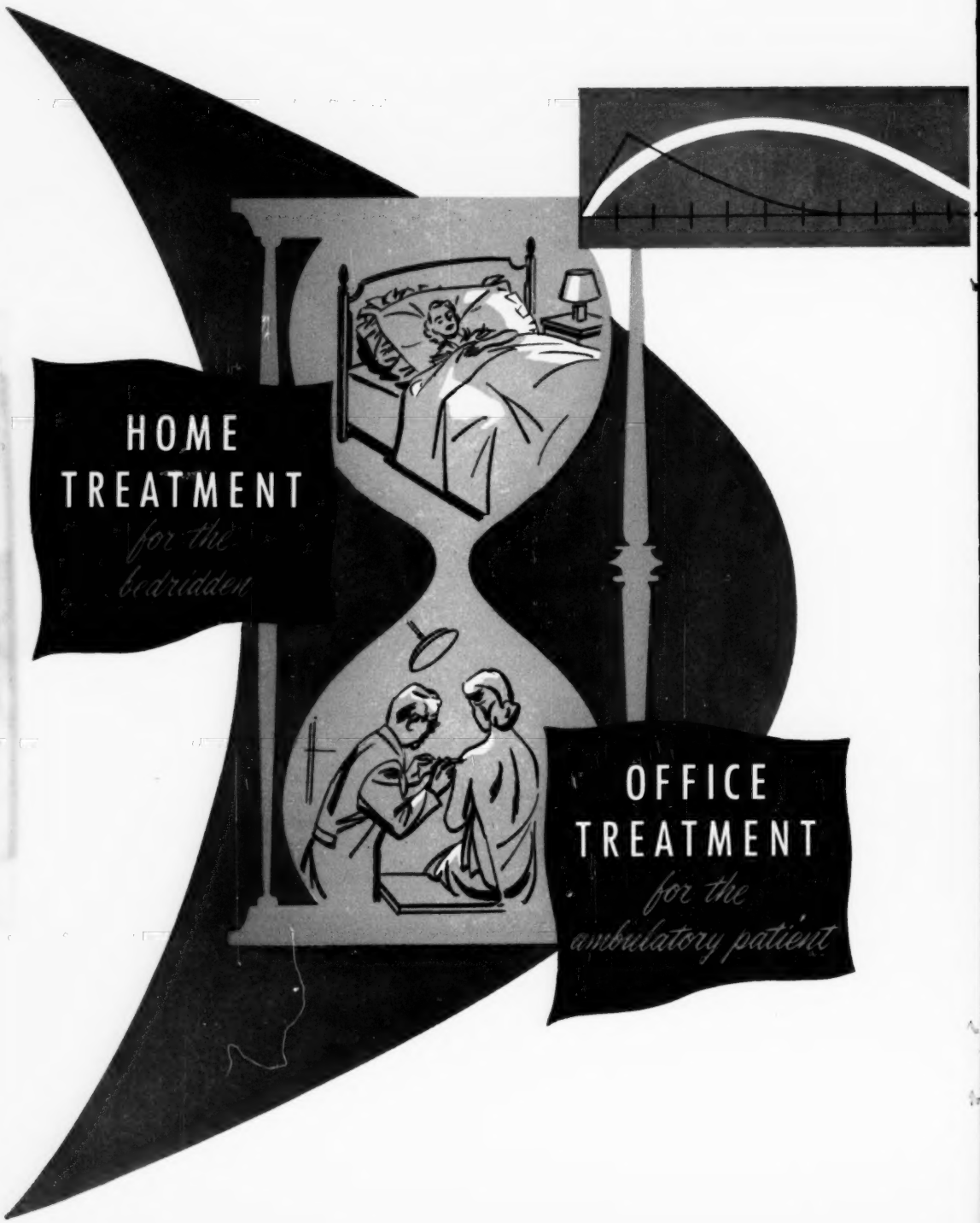
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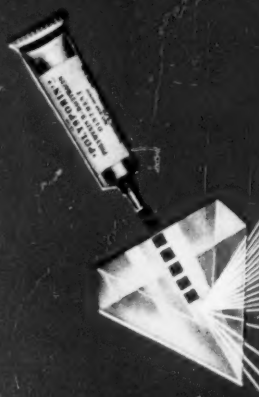

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HEMODYNAMIC ALTERATIONS IN ACUTE MYOCARDIAL INFARCTION. I. CARDIAC OUTPUT, MEAN ARTERIAL PRESSURE, TOTAL PERIPHERAL RESISTANCE, "CENTRAL" AND TOTAL BLOOD VOLUMES, VENOUS PRESSURE AND AVERAGE CIRCULATION TIME [1, 2]

By EDWARD D. FREIS, HAROLD W. SCHNAPER, ROBERT L. JOHNSON,[3] AND GEORGE E. SCHREINER

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(Submitted for publication June 18, 1951; accepted August 20, 1951)

Despite its importance little is known of the hemodynamic abnormalities which accompany acute myocardial infarction in man. Aside from clinical observations, the measurement of venous pressure (1) and total blood volume (1, 2), few systematic studies have been carried out in clinical cases. The reason for this has been the impossibility of subjecting such acutely ill patients to difficult or time-consuming technical procedures. However, recent studies (3, 4) indicated that the values for cardiac output obtained with the Hamilton dye injection method were not significantly different from those obtained with the intravenous catheterization method using the Fick principle. It seemed probable, therefore, that the Hamilton dye method suitably modified could be used to determine cardiac output at the bedside in acutely ill patients. This report describes the changes observed using the dye injection method and other procedures in a series of cases with myocardial infarction as compared to a group of normal subjects and hypertensive patients.

METHODS

Fourteen hemodynamic studies were carried out in 11 patients with acute myocardial infarction, eight determinations in seven normal male medical students, and five in a similar number of hypertensive patients. It was essential in the cases of acute myocardial infarction to carry out the experimental procedures rapidly at the bedside and without serious disturbance to the patient. For this reason the dye injection method of Hamilton and colleagues (5) as modified by Werko and associates (4) was used for determining cardiac output. After novocainizing the skin and making a small incision through the dermis with a scalpel blade, a 15 gauge Robb type needle 5 cm. in length [4] was inserted into the femoral artery. This permitted excellent flows of 2 to 3 cc. of blood per second while the rate of flow could be controlled by the stopcock which forms a part of the hub of this needle. Despite the large size there was little difficulty with hematoma formation when the puncture site was compressed for 20 minutes after removing the needle.

The dye was injected into the central circulation by means of polyethelene tubing (1.12 mm. inner diameter),[5] previously sterilized by soaking in 1 to 1,000 aqueous zephiran solution. After nicking the novocainized skin over an antecubital vein situated on the medial aspect of the arm, a 13 gauge needle was introduced into the vein and using sterile precautions the polyethelene tubing was inserted through the needle to a level calculated to be midway between the axilla and the center of the sternum. The catheter always was advanced several inches further and then retracted to be certain that it was not in a venous tributary. Since the placement could not be determined by fluoroscopy it was decided not to attempt to reach the right auricle since in the process the catheter might pass over into the jugular or opposite subclavian veins. Following placement of the catheter the 13 gauge needle was removed and an 18 gauge needle inserted into the end of the catheter a distance of 1 inch.

Every effort was made to carry out these procedures calmly and quietly and towels were so draped that the patient could not see the insertion of the needles. The "team" of investigators was trained so that two men

[1] Supported in part by research grants from the National Heart Institute, U. S. Public Health Service, The Squibb Institute for Medical Research, New Brunswick, N. J. and Irwin Neisler & Co., Decatur, Ill.

Presented in part at the Southern Society for Clinical Research, Jan. 19, 1952.

[2] Sponsored (in part) by the VA and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[3] Captain, MC, U.S.A.F.

[4] Becton, Dickinson & Company, Rutherford, N. J.

[5] Clay-Adams Company, Inc., New York, N. Y.

would insert the catheter while another placed the Robb needle in the femoral artery. After some practice these procedures could be carried out in less than 10 minutes. Following this, the central venous pressure was measured through the catheter using a water manometer and the arterial pressure was measured using a strain gauge manometer,[6] a carrier-wave type amplifier[7] and single channel recorder.[8] Mean arterial pressure was determined by electronic integration of the pulse wave.

The injection of T-1824[9] in 0.5 per cent solution and collection of samples from the femoral artery were carried out according to method of Werko and associates (4). Following this the arterial pressure was measured again and additional arterial samples were withdrawn at 10, 15, and 20 minutes for determination of total plasma volume. In order to minimize hemolysis all samples were collected in parafined tubes containing a drop of dried heparin. Hemolyzed samples (rarely obtained) were discarded. The average circulation time and central blood volume were determined according to the method of Hamilton and co-workers (5). It was recognized that this method was not precise because of the variable contribution of blood from other large veins entering the right heart (6). However, it seemed that large discrepancies could be determined by this method especially since comparisons were made with normal and hypertensive controls who were studied using the same methods.

RESULTS

It was apparent that the hemodynamic changes found in cases of myocardial infarction varied with the clinical state of the patient. For this reason the cases have been divided into three groups: (*1*) mild, (*2*) moderately severe and severe and (*3*) cardiogenic "shock." The basis of such differentiation was made on clinical grounds. The mild cases were those who exhibited little or no evidence either of shock or of congestive heart failure and made a rapid and uneventful recovery. The moderately severe and severe group exhibited definite evidence of circulatory embarrassment, particularly prostration, and manifestations of congestive heart failure. The cardiogenic shock cases all were severely ill exhibiting extreme prostration, frequently with mental confusion as well as the clinical appearance of shock. All of the latter cases terminated fatally during the acute episode.

[6] Model P-23A, Statham Laboratories, Beverly Hills, Calif.

[7] Designed and built by Mr. Robert Wood, Tacoma Park, Md.

[8] Visocardiette, Sanborn Co., Boston, Mass.

[9] William Warner Co., New York, N. Y.

Mean Arterial Pressure, Cardiac Output, Heart Rate, Stroke Volume and Total Peripheral Resistance

In the normal group the mean pressure in the femoral artery ranged from 63 to 93 mm. Hg (average 84 mm. Hg), while in the hypertensive patients the average was 145 mm. Hg with a range of 104 to 189 mm. Hg (Table I). The mean arterial pressures in the cases with myocardial infarction were as follows: in the mild group the average was 95 mm. Hg (range 85 to 113 mm. Hg), in the moderately severe and severe cases it was 89 mm. Hg (range 68 to 120 mm. Hg) and in the patients with cardiogenic shock the average was 79 mm. Hg (range 73 to 88 mm. Hg). Extreme hypotension was not observed in the patients with coronary occlusion even in those cases who appeared clinically to be in "shock" and who later died.

Cardiac outputs were unusually high in the normal subjects, the mean cardiac index being 4.0 ± 0.81 L. per min. (Table I). The high values probably were due to the fact that all were healthy, young male individuals. These subjects were not unduly apprehensive as evidenced by the average heart rate of 64 per minute. In the hypertensive patients, who were more comparable in age to the cases with myocardial infarction, the mean cardiac index was 3.2 ± 0.4 L. In the patients with coronary occlusion the cardiac output was within the normal range in the mild cases, but was abnormally low in the cases exhibiting shock. The mean cardiac index in the mild group was 3.4 ± 0.8 L. per min., in the moderately severe and severe patients 2.9 ± 0.3 L. per min., and in the shock group 1.8 ± 0.4 L. per min.

The heart rate usually was increased in the patients with myocardial infarction but there was considerable variation from patient to patient. The average in the mild cases was 92 beats per minute and the range 64 to 124. In the moderately severe and severe group the heart rate ranged between 85 and 122 per minute (average 101), and in the cases exhibiting shock, it ranged from 74 to 160 per minute with an average of 128. Thus, in general the heart rate increased with increasing severity of the infarction.

The stroke volume was unusually high in the normal subjects averaging 118 ± 16 cc. In the

TABLE 1

Hemodynamic data in normal subjects, hypertensive cases and patients with acute myocardial infarction of varying degrees of severity

Pt.	Age (yrs.)	Surface area (sq. m.)	Mean arterial pressure (mm. Hg)	Cardiac output (liters per min.)	Cardiac index	Heart rate (per min.)	Stroke volume (cc.)	Total peripheral resistance (dynes / cm.$^{-5}$ sec.)	"Central" venous pressure (mm. H_2O)	"Central" blood volume (liters)	Total blood volume (liters)	Total blood volume (liters per sq. m.)	Average circulation time (sec.)	Time after infarction (days)	Outcome *
Normal young male subjects															
R. B.	29	1.72	83	9.1	5.3	70	130	725	88	2.1	5.6	3.3	13.9		
J. D.	26	1.96	93	8.9	4.6	60	149	775	68	3.4	8.2	4.2	23.2		
W. B.	28	1.92	63	7.0	3.7	68	103	725	76	2.5			21.1		
J. A.	21	1.84	88	8.1	4.4	66	123	875		2.9	5.6	3.1	21.5		
C. L.	26	2.09	84	7.2	3.4	68	105	950		2.3	5.0	2.4	19.7		
J. L.	22	1.87	78	8.9	4.8	73	122	700		4.5	6.1	3.3	30.4		
H. Bi.	36	1.89	91	5.2	2.8	54	98	1,386		2.3	4.9	2.8	26.5		
H. Bi.	36	1.89	94	6.2	3.3	56	110	1,200	56	2.1	5.6	3.0	20.4		
Mean and S.D.	28	1.90	84 ± 10	7.6 ± 1.35	4.0 ± .81	64	118 ± 16	925 ± 225	72	2.8	5.9	3.2	22.1 ± 4.5		
Hypertensive cases															
E. J.	47	1.77	104	4.5	2.5	62	73	1,850		2.2	6.9	3.9	28.8		
A. M.	58	1.84	155	6.0	3.2	100	60	2,000	50	1.9	5.9	3.2	19.5		
M. F.	61	1.82	189	6.8	3.7	79	86	2,225		3.0	5.7	3.1	26.6		
B. L.	45	1.86	120	5.7	3.1	94	61	1,450		2.8	4.9	2.6	29.0		
A. F.	29	2.03	159	6.9	3.4	83	83	1,835		2.3	6.4	3.1	20.2		
Mean and S.D.	48	1.86	145 ± 30	6.0 ± .87	3.2 ± .4	83	73 ± 11	1,875 ± 250	50	2.4	6.0	3.2	24.8 ± 4.2		
Myocardial infarction patients															
Mild															
H. Bo.	60	2.14	95	6.3	2.9	64	98	1,225		3.5			33.4	1	R.
L. B.	57	1.90	86	5.3	2.8	67	79	1,300	117	3.1	6.4	3.4	34.7	1	R.
J. W.	45	1.83	87	6.0	3.3	124	49	1,150	84	1.8	4.7	2.6	18.3	1	R.
J. S.	47	1.85	113	8.7	4.7	114	76	1,050	70	2.2	5.3	2.9	15.3	0.5	R.
Mean and S.D.	50	1.93	95 ± 11	6.6 ± 1.3	3.4 ± .76	92	76 ± 14	1,175 ± 100	90	2.7	5.5	3.0	25.4 ± 8.7		
Moderately severe and severe															
J. V.	54	1.91	103	5.0	2.6	103	36	1,650	144	2.4	6.2	3.5	29.6	3	R.
T. P.	63	1.80	78	4.8	2.7	85	57	1,300		3.4	4.3	2.4	41.6	1	R.
D. S.	64	1.80	75	5.1	2.9	86	60	1,175	160	2.9	5.2	2.8	33.8	0.5	D., 3 days later
R. E.	56	1.96	68†	5.2	2.7	108	48	1,000	81	2.8	4.7	2.4	32.0	1	R.
F. McC.	58	1.82	120	6.2	3.4	122	51	1,550	84	3.2	5.6	3.1	31.2	1	D., 11 days later
Mean and S.D.	59	1.86	89 ± 20	5.3 ± .5	2.9 ± .31	101	50 ± 9	1,325 ± 250	120	2.9	5.2	2.8	33.6 ± 4.1		
Cardiogenic "shock"															
F. McC.	58	1.82	72	4.4	2.4	160	28	1,300	115	2.0‡	6.4	3.5	27.3‡	3	D., 9 days later
P. C.	57	1.78	83	2.7	1.5	74	37	2,450	90	3.5			78.8	0.5	D., 7 days later
G. McK.	54	1.89	88	3.0	1.6	140	22	2,350		1.8	4.3	2.3	35.3	0.5	D., 6 days later
G. McK.	54	1.89	73	2.8	1.5	140	20	2,100	140	1.7	4.6	2.4	36.4	2	D., 4 days later
Mean and S.D.	56	1.84	79 ± 11	3.2 ± .72	1.8 ± .37	128	27 ± 7	2,050 ± 450	115	2.3	5.1	2.7	44.5 ± 21		
Mean for all infarctions	55		88	5.0	2.7	106	51	1,575	108	2.6	5.2	2.9	34.4		
J. V. (after recovery)	54	1.80	74	5.8	3.3	80	73	1,020	74	2.6	6.1	3.4	24.9	88	

S.D. (standard deviation) = $\sqrt{\frac{\Sigma d^2}{n}}$.

* R. signifies recovered while D. indicates death of the patient.

† Patient's blood pressure remained low following recovery. He did not present the clinical picture of shock.

‡ Values not comparable with prior data on this patient since in the second determination it was impossible to insert the catheter in an arm vein; it was inserted in the femoral vein and estimated by distance to be in the right auricle.

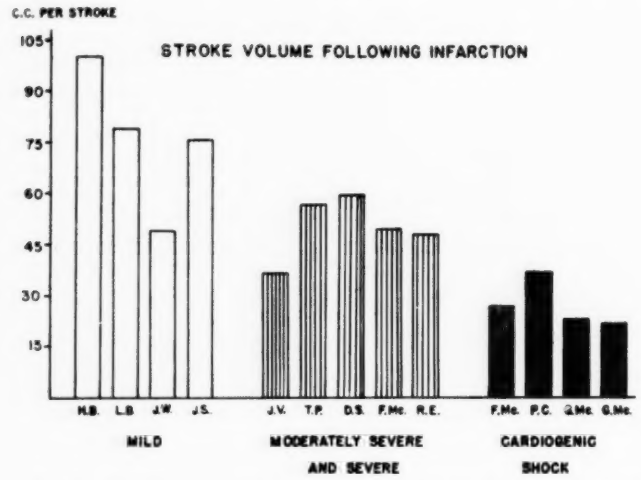

FIG. 1. RELATIONSHIP BETWEEN SEVERITY OF DISEASE AND DEGREE OF REDUCTION OF STROKE VOLUME IN THE PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

hypertensives the mean stroke volume was 73 ± 11 cc. and in the patients with myocardial infarction of mild degree the mean was 76 ± 14 cc. Stroke volume was slightly reduced in the moderately severe and severe group averaging 50 ± 9 cc., while in the patients with the shock syndrome there was a marked reduction of stroke volume to 27 ± 7 cc. (Table I, Figure 1).

The mean total peripheral resistance for the normal subjects was 925 ± 225 dynes cm.$^{-5}$ sec. and for the hypertensives was 1,875 ± 250 dynes cm.$^{-5}$ sec. In the patients with myocardial infarction of mild degree the total peripheral resistance was 1,175 ± 100 dynes cm.$^{-5}$ sec. However, it was slightly increased in the moderately severe and severe group being 1,325 ± 250 dynes cm.$^{-5}$ sec. and strikingly increased in the cardiogenic shock cases, averaging 2,050 ± 450 dynes cm.$^{-5}$ sec.

"Central" Venous Pressure, Average Circulation Time, "Central" Blood Volume and Total Blood Volume

The "central" venous pressure measured in the region of the subclavian or innominate veins averaged 90 mm. H_2O (range 70 to 117 mm.) in the patients with mild coronary occlusion. In the patients with moderately severe or severe myocardial infarction the venous pressure varied between 81 and 160 mm. H_2O (average 120 mm. H_2O). The average venous pressure for the cardiogenic shock cases was 115 mm. H_2O (range 90 to 140 mm.).

The average circulation time was 22.1 ± 4.5 sec. in the normal subjects, 24.8 ± 4.2 sec. in the hypertensive cases, and 25.4 ± 8.7 sec. in the patients with myocardial infarction of mild degree. The circulation time was prolonged in the more severe cases being 33.6 ± 4.1 sec. in the patients with moderately severe or severe attacks, and 44.5 ± 21 seconds in the cardiogenic shock group.

The "central" blood volume was not significantly different in the various groups, the average values being as follows: 2.8 L. in the normal subjects, 2.4 L. in the hypertensive patients, 2.7 L. in the patients with mild coronary occlusion, 2.9 L. in the moderately severe and severe group, and 2.3 L. in the patients with cardiogenic shock.

The total blood volume tended to be slightly reduced in the cases of severe myocardial infarction but the differences were not sufficiently marked to be significant in view of the small series of patients and there was considerable overlapping of individual values. The total blood volume ranged between 2.3 and 4.2 L. per sq. m. surface area (average 3.2 L.) in the normal subjects. In the hypertensives the average was 3.2 L. and the range

2.6 to 3.9 L. The total blood volumes in the patients with myocardial infarction were as follows: average 3.0 and range 2.6 to 3.4 L. per sq. m. in the mild group, average 2.8 and range 2.4 to 3.5 L. in the moderately severe and severe group, and average 2.7 and range 2.3 to 3.5 L. per sq. m. in the cardiogenic shock patients.

In patient F. McC. the typical picture of cardiogenic shock developed three days after the infarction and two days after the original studies (Table I and Figure 2). Hence, it was possible to compare the values obtained prior to and after the development of the shock-like state. In the interval between the tests, the electrocardiogram indicated extension of the infarct while the arterial pressure had fallen from hypertensive to hypotensive levels. The most striking change in this patient was the reduction of stroke volume from 51 to 28 cc. The venous pressure increased from 84 to 115 mm. H_2O and the heart rate from 122 to 160 beats per minute. Unfortunately during the shock phase it was impossible to thread the catheter into an arm vein. The femoral vein was entered and the catheter tip placed in the neighborhood of the right auricle. Hence, the measurements of average circulation time and central blood volume were not comparable in the two determinations.

In another patient (J. V.) the determinations were repeated approximately two months after the myocardial infarction when he had completely recovered (Table I). This patient was critically ill during the acute episode manifested by pulmonary edema, dyspnea and orthopnea. Following recovery the mean arterial pressure fell markedly from 103 to 74 mm. Hg while the cardiac output rose slightly from 5.0 to 5.8 L. per minute. The stroke volume, however, increased markedly from 36 to 73 cc. This was due in most part to the marked elevation of heart rate to 140 beats per minute during the acute episode which fell on recovery to 80 per minute. There also was a striking reduction in total peripheral resistance from 1,650 to 1,020 dynes cm.$^{-5}$ sec. The venous pressure also fell from 144 to 74 mm. H_2O, while the average circulation time became somewhat reduced from 29.6 to 24.9 seconds. However, despite the high venous pressure and evidence of cardiac failure at the time of the acute attack, the central blood volume was not significantly changed, being 2.4 L. initially and 2.6 L.

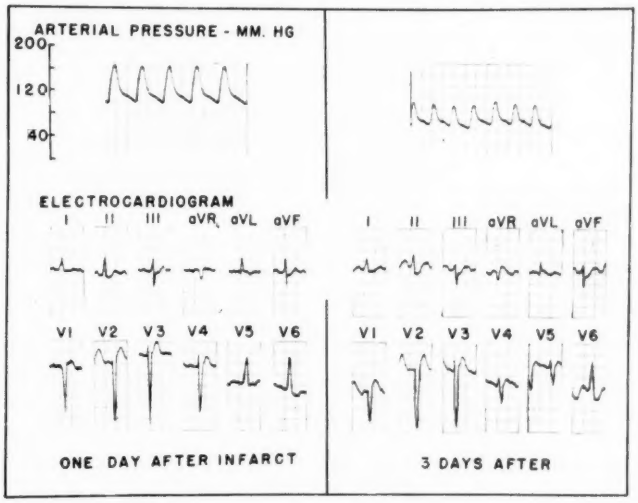

FIG. 2. CUTTINGS TAKEN FROM TRACINGS OF FEMORAL ARTERIAL PRESSURE AND ELECTROCARDIOGRAMS IN PATIENT F. MCC., A WHITE MALE, AGE 58 YEARS, WITH ACUTE MYOCARDIAL INFARCTION

During the interim between the first and second determinations the patient developed the clinical picture of "shock." See text for further details.

after recovery. The total blood volume also remained essentially unchanged.

DISCUSSION

The disturbances in cardiovascular hemodynamics observed in the patients with myocardial infarction varied with the severity of the attack. The mild cases failed to exhibit significant abnormalities. In the patients with cardiogenic shock the most marked disturbances were the serious reduction of the stroke volume of the heart and the marked increase in total peripheral resistance. The moderately severe and severe cases formed an intermediate group between the mild and the shock patients so far as hemodynamic abnormalities were concerned. Thus, the severity of the attack as judged by clinical observations appeared to be correlated directly with the degree of depression of stroke volume.

Since the central blood volume was not significantly reduced it seemed reasonable to conclude that the depression of stroke output was due to failure of the heart as a pump and not to hypovolemia. These observations are in agreement with similar data obtained by Mendlowitz, Schauer and Gross (7) in dogs subjected to ligation of coronary arteries. In addition, Gilbert, Aldrich and Anderson, utilizing techniques similar to those used in the present study, found a reduction of cardiac output in two of three patients with acute myocardial infarction (8).

On the basis of the observed data the sequence of hemodynamic events following severe myocardial infarction appeared to be as follows: if the infarction was extensive or if it involved a previously damaged myocardium there was diminished contractility and hence a failure of stroke output. The consequent reduction of arterial pressure immediately resulted in the activation of neurogenic reflexes and possibly other compensatory mechanisms which produced an increase in total peripheral resistance and tachycardia. Thus, the hemodynamic response was not unlike the compensated phase of hypovolemic shock (9), except that the disturbance was due to ineffectual contraction of the myocardium rather than to blood loss.

This interpretation of the hemodynamic alterations accompanying myocardial infarction are in essential agreement with the classical studies of Wiggers and his colleagues on experimental coronary occlusion in animals. In his review of this work (10) Wiggers points out that the infarcted segment balloons out rather than contracts during systole. In some animals this is compensated for by an increase in diastolic ventricular volume with stretching of the muscle fibers and hence an increased contraction of the uninvolved portions of the myocardium. In others, however, this compensatory mechanism is insufficient to restore the cardiac output to normal. In these cases the arterial pressure falls progressively while the ventricles, atria, pulmonary vessels, and veins fill with blood.

Wiggers further states that, "the similarity of clinical syndromes following coronary occlusion and loss of blood or plasma is due to the fact that in both cases, cardiac output is decreased, but for different reason, hypovolemia in the case of shock due to blood loss and failure of the contractile capacity of the ventricle in the case of myocardial infarction. One of the consequences of lowering arterial pressure is that moderator vascular reflexes operate to cause generalized vasoconstriction . . . and constriction of skin reservoirs in cases of coronary occlusion . . . merely tends to intensify the engorgement and adds to the volume of blood that the defective myocardium cannot move."

Unlike the condition seen in congestive heart failure, the total blood volume tended to be slightly reduced and the central blood volume was not increased. This occurred even in those patients with elevations of venous pressure and, hence, the latter phenomenon cannot be ascribed to heart failure alone. The venous pressure, however, appeared to correlate with the total peripheral resistance, usually being high in the patients with elevated total peripheral resistances. These observations suggest that in addition to peripheral arterial constriction there also was venoconstriction. Elevation of venous pressure can be obtained in normal individuals following drugs which increase total peripheral resistance such as angiotonin (11) and norepinephrine (12). It seems not unlikely, therefore, that the increase in venous pressure observed in coronary occlusion is at least in part a reflection of the overall increase of total peripheral resistance.

It was surprising to find that the mean pressure as measured in the femoral artery was not usually significantly below normal in these patients, many of whom appeared to be in shock

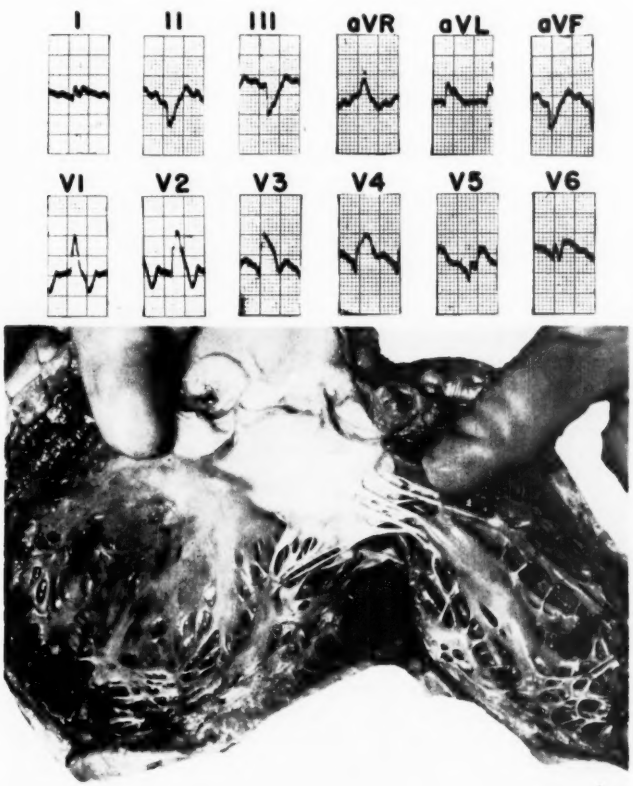

FIG. 3. CUTTINGS FROM ELECTROCARDIOGRAM AND PHOTOGRAPH OF THE HEART OF PATIENT G. MCK., A WHITE MALE, AGED 54 YEARS WHO EXHIBITED CARDIOGENIC SHOCK

The electrocardiogram is consistent with a diagnosis of acute anteroseptal infarction and intraventricular block, R.B.B.B. type. The photograph discloses extensive infarction and thinning of the left ventricular myocardium and "ballooning" of the intraventricular septum into the cavity of the right ventricle. See Table I for hemodynamic data.

clinically in that they were pale, mentally dulled and had weak and rapid pulses. Yet most of the patients who were judged to be in shock by clinical observations exhibited pressures in the femoral artery not markedly lower than in the normal subjects. The mean pressure as recorded directly from the femoral artery in shock due to skeletal trauma or blood loss is much lower, varying between 40 and 60 mm. Hg depending on the severity of the shock (13), as compared to an average value of 79 mm. Hg in the present series diagnosed clinically as being in cardiogenic "shock."

However, the level of arterial pressure probably was maintained at barely normal levels by means of the marked increase in total peripheral resistance. If the stroke volume fell further or persisted at low levels for long periods, eventually a limit would have been reached when the compensatory mechanisms of vasoconstriction and tachycardia could no longer compensate. This event apparently occurred in the case of G. McK. whose initial determination soon after the infarction revealed a mean arterial pressure of 88 mm. Hg despite a stroke volume of only 22 cc. However, two days later the mean arterial pressure had fallen to 73 mm. Hg with a stroke volume of 20

cc. It would appear in this case that the compensatory mechanisms began to fail after several days. A similar sequence seemed to have occurred also in patient F. McC.

One of the most puzzling aspects of the shock-like state accompanying acute myocardial infarction has been the presence on the one hand of the clinical features of hypovolemic shock, and on the other, of hypervolemic heart failure. Thus, the patient may exhibit at the same time pallor, moderate hypotension, and a thready pulse and also may have a high venous pressure, pulmonary edema, dyspnea and orthopnea. These discrepancies, however, can be fitted into a comprehensive schema. It is well known that if in hypovolemic shock the combination of low stroke volume and intense peripheral vasoconstriction is allowed to persist, there is fluid retention and eventually edema, *i.e.*, the so-called lower nephron nephrosis syndrome (14) associated with a reduction of renal blood flow and glomerular filtration rate (15).

It seems possible that a similar sequence may occur in acute myocardial infarction. Initially in this condition there is a shock-like state whereas the signs of congestive heart failure occur characteristically several days following the infarction (1). This pattern is similar to that observed in the lower nephron syndrome. However, in the patients with coronary occlusion, the signs of congestive heart failure are greatly exaggerated because of defective myocardial contractility. The administration of blood or plasma to such patients should only add to the insult since there is no deficiency of central blood volume but rather a failure of output. In G. McK., an attempt was made to transfuse the patient intra-arterially. The rapid administration of blood resulted almost immediately in increased dyspnea and substernal pain. The transfusion was stopped and the patient recovered. Further attempts at transfusing even small amounts of blood produced the same symptoms and, after 200 cc. had been administered, the attempt to transfuse this patient was abandoned. Several other subjects claimed that they derived definite relief of substernal pain and dyspnea following the cardiac output determination which entailed the withdrawal of 100 to 150 cc. of blood.

These observations are in agreement with those of Boyer who in a review of cardiogenic shock (16) concluded that, "The bulk of evidence both direct and theoretical is overwhelmingly in favor of the concept that shock in myocardial infarction is largely, and probably solely, a manifestation of heart failure. . . . Indeed pulmonary edema may constitute a more immediate threat to life (by lowering the oxygen content of the blood) than does shock, and venesection may occasionally be life-saving." Stead and Ebert withdrew 500 and 650 cc. of blood, respectively, from two patients with myocardial infarction and shock with no evident harmful effects (17).

The hypovolemia and terminal heart failure observed in animals in the late stages of hemorrhage shock (18) no doubt lead to an insufficient coronary circulation as pointed out by Corday and his co-workers (19), but is not at all comparable to the hemodynamic situation existing in myocardial infarction. Corday and associates ligated coronary vessels and found that if the animals were then bled to shock levels, the non-contractile zone of myocardium became larger. Transfusion in this instance improved the contractility of the ischemic area. However, this experimental situation cannot be directly related to the patient with coronary occlusion since as determined by the present study there is no deficiency of central blood volume but rather a failure of the ventricle to expel the normal amount of blood presented to it.

The compensatory mechanisms activated by the reduction of stroke volume aid in the maintenance of aortic pressure and hence of coronary blood flow; but they also may have harmful consequences on the course of the disease, by adding extra burdens to an already damaged heart. Such deleterious influences include (*1*) an elevation of total peripheral resistance thereby increasing the work of the heart, (*2*) tachycardia which also adds to the work of the heart and results in a less effective stroke output, (*3*) increased venoconstriction resulting in increased venous filling pressure in the presence of a failing heart, and (*4*) a fluid retention syndrome secondary to renal vasoconstriction. It seems possible that such a series of compensatory reactions teleologically designed to tide a wounded animal over an emergency situation may be harmful in the patient with a weakened myocardium due to coronary occlusion.

SUMMARY AND CONCLUSIONS

The hemodynamic abnormalities associated with severe myocardial infarction were a reduction of cardiac output, especially stroke volume, an increase of total peripheral resistance, heart rate, central venous pressure and average circulation time, a slight and possibly insignificant reduction of total blood volume and an essentially normal central blood volume. The less severe cases did not exhibit these abnormalities.

It is suggested that the initial change after an extensive infarction is a reduction of stroke volume due to the myocardial insult. This is followed by a series of compensatory mechanisms characterized by generalized, intense vasoconstriction probably including venoconstriction, and by tachycardia producing a clinical picture resembling shock. Such intense vasoconstriction in the presence of a reduced stroke volume may be followed by a fluid retention syndrome which, in the presence of a defective myocardium, results in congestive heart failure as well as the shock-like state. These compensatory reactions to a reduced stroke volume appear beneficial in that by raising aortic pressure they aid in the maintenance of coronary blood flow, but they also seem detrimental in that they add in various ways to the work load of an already damaged heart.

CASE SUMMARIES

H. B.—A 60 year old white male dentist developed increasing substernal oppression seven hours prior to admission to the hospital. This was associated with profuse sweating but there was no dyspnea. The lungs were clear and the heart sounds were of good quality. The electrocardiogram was within normal limits on the day of admission but the next day disclosed changes consistent with a recent posterior myocardial infarction. Recovery was uneventful.

L. B.—A 56 year old colored male was admitted to the hospital complaining of substernal pain of three hours duration. He had been hospitalized two years previously for a "heart attack." The lungs were clear and there was no dyspnea or orthopnea. The electrocardiogram was consistent with a recent posterior myocardial infarction. Recovery was uneventful.

J. W.—This 45 year old white male had experienced angina intermittently for two months. On the day of admission while pushing his car he developed substernal pain lasting for six hours. Aside from apprehension the physical examination was not remarkable. The electrocardiogram disclosed the pattern of fresh anterolateral myocardial infarction. He made an uneventful recovery.

J. S.—A 37 year old male developed severe squeezing pain in the substernal area 11 hours before admission to the hospital. For the two months preceding this episode there had been angina on exertion. The patient was pale but the heart sounds were of good quality and the lungs were clear. The electrocardiogram indicated a recent anteroseptal infarction. Recovery was uneventful.

J. V.—This 54 year old male with diabetes mellitus, dyspnea and angina on effort, all of one year's duration, began to have intermittent and then continuous substernal pain radiating to both arms on the day preceding admission to the hospital. His early clinical course was characterized by marked dyspnea and orthopnea with moist rales at the lung bases, but no hepatomegaly or peripheral edema. Serial electrocardiograms were consistent with a diagnosis of recent, subendocardial, anterolateral infarction as well as old anterolateral infarction. The patient began to improve after the third hospital day and thenceforth made an uneventful recovery.

T. P.—A 63 year old male, who had suffered with asthma for six years, developed paraesthesias of the left arm and hand for three weeks and severe continuous substernal pain without radiation for one day prior to admission to the hospital. The patient was dyspneic, mentally dulled and exhibited crackling rales at both lung bases. The electrocardiogram indicated anteroseptal infarction. Several days following admission the patient developed a hemiparesis, which, however, gradually cleared and the further course was uneventful.

D. S.—This 64 year old male developed diabetes mellitus one year ago at which time the electrocardiogram was interpreted as indicating "coronary insufficiency." Two days prior to admission to the hospital he developed crushing substernal pain radiating to the left shoulder. There was slight dyspnea but the lungs were clear. The electrocardiogram indicated recent posterior infarction and AV Block with *2* to 1 conduction. On the second hospital day he developed hypotension, bradycardia and oliguria and died on the following day. Autopsy was not obtained.

R. E.—This 56 year old male, who had mild essential hypertension and diabetes mellitus for many years, suffered a posterior myocardial infarction three years ago. He was admitted to the hospital because of an attack of pulmonary edema. Response to therapy was slow and while convalescing he developed typical anginal pain and a recurrence of congestive heart failure. The electrocardiogram indicated a recent anteroseptal infarction. The hemodynamic studies were carried out on the second day of this attack. The patient appeared to recover from this episode but died suddenly five weeks later during a third attack of angina and acute pulmonary edema.

F. McC.—This 58 year old male had experienced angina on effort for the past six months. On the evening of admission to the hospital he developed squeezing substernal pain accompanied by sweating and dyspnea. There were fine moist rales at both bases and a pleuropericardial friction rub was heard. The electrocardiogram disclosed posterior and lateral subendocardial infarction. Three days after admission he developed hypotension, extreme prostration, cyanosis, increased pulmonary rales,

hepatomegaly and oliguria but no orthopnea. He remained in this state for an additional 10 days, the blood non-protein nitrogen rising gradually during this period to a level of 210 mg. per cent two days before death. No autopsy was performed.

P. C.—A 57 year old male suffered a cerebrovascular accident two years ago and an acute myocardial infarction one year ago. Since the latter he had frequent bouts of angina particularly at night. On the day of admission he developed mild but continuous pain in the parasternal area. On admission he appeared pale with cold hands and feet and was dyspneic and orthopneic. The heart was enlarged, the lungs moderately congested and the liver edge was palpated 4 cm. below the costal margin. The electrocardiogram revealed the pattern of fresh anterolateral infarction. The blood pressure remained low, the congestive heart failure increased and the patient died after one week in the hospital. Permission for autopsy was refused.

G. McK.—A 54 year old male who previously had been in excellent health developed transient substernal pain two months before admission to the hospital. On the morning of admission he was awakened with severe substernal pain radiating to the left arm accompanied by profuse sweating but no dyspnea. On admission he appeared to be in shock with distant heart sounds and fine moist rales at the lung bases. The electrocardiographic changes indicated acute anteroseptal infarction, intraventricular block (RBBB type) and frequent interpolated ventricular beats. The clinical course was characterized by increasing shock with mental confusion and later delirium. The signs of congestive heart failure, except for dyspnea, never were prominent and there was no orthopnea. The blood nonprotein nitrogen rose gradually to 137 mg. per cent and he died six days after admission. Autopsy revealed coronary thrombosis left and infarction of the entire wall of the left ventricle extending into the interventricular septum. There was also chronic passive congestion of the lungs, liver, spleen and gastrointestinal tract.

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THE DETERMINATION OF THYROIDAL AND RENAL PLASMA I^{131} CLEARANCE RATES AS A ROUTINE DIAGNOSTIC TEST OF THYROID DYSFUNCTION [1, 2]

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INTRODUCTION

Since the early investigations of Hertz, Roberts and Evans (1) and Hamilton (2–4), various methods of estimating the radioiodine accumulating capacity of the thyroid gland have been employed as diagnostic clinical tests as well as in physiological studies. The most common procedures have involved the determination of the 24 hour thyroid uptake (5–7) or the total 24, 48, or 72 hour urinary excretion (8–10) of radioiodine following its oral administration. While these methods have provided an additional diagnostic tool for the evaluation of thyroid function, equivocal results have been obtained in about 10% of the cases in most series. Furthermore, where renal function is impaired, values characteristic of hyperthyroidism have been observed in the presence of normal thyroid function (10).

Measurements of the rate of disappearance from the blood (11) or the rate of urinary excretion (12, 13) or uptake by the thyroid (13–15) of radioiodine have proved to be involved procedures and have not satisfactorily eliminated overlapping, although the frequency of borderline cases has been reduced.

The relative and indirect nature of all these methods has been emphasized in a recent review (16) and in other reports (14) by the group at the Mayo Clinic. They have concluded (16), in agreement with Myant, Pochin and Goldie (17), that the clearance of I^{131} from the blood by the thyroid is probably the most reliable index of the iodine accumulating function of the gland. Present techniques, however, have made this determination a complicated procedure, precluding its more widespread use.

It is the purpose of this paper to present a simple, expedient method of obtaining the thyroidal and renal plasma iodide clearances without the necessity of performing analyses of blood samples.

Studies of the clearance rates in 87 euthyroid, 18 hyperthyroid and five hypothyroid subjects and a comparison with the 24 hour thyroid uptake and renal excretion values are presented. The significance of these studies in relation to the results obtained by other methods of measurement of the iodine accumulating capacity of the thyroid is discussed.

METHODS

Subjects of the study were patients of the Veterans Administration Hospital, Bronx, New York. All cases in which there was a history of recent iodine administration in any form were excluded from the study.

For routine diagnostic testing, doses of 25–50 μc carrier free I^{131} as KI in 2–8 ml of sterile isotonic saline solution were administered intravenously over a 10–25 second period. Calibration of the syringes revealed an accuracy of $\pm 3\%$ or better. For the special time-concentration studies, doses of 100–750 μc were administered. Doses of more than 150 μc were restricted to patients with carcinoma or with hyperthyroidism for whom the therapeutic use of I^{131} was planned.

The Geiger counters were standardized for I^{131} against a Braestrup type ionization chamber which was initially calibrated in terms of the "New York millicurie" which at the present time is equal to 0.85 Oak Ridge millicurie.

In vivo counting over the thyroid was performed with an RCL bismuth gamma counter, which at nine inches from the thyroid, had a sensitivity of 105 C/M/μc I^{131} over a background of approximately 60 C/M, as determined by calibration against a dummy source of known activity immersed in a paraffin filled phantom neck. The counter tube was attached to a scaling circuit whose output fed into a Streeter-Amet register, which automatically printed the cumulative counts at one minute intervals. The arrangement and shielding of the apparatus is shown in Figure 1. Because of the wide window in the counter shield and long sensitive region of the tube (five inches),

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the results of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] Presented, in part, at the Clinical Research Meeting, New York Academy of Medicine, April 5, 1951.

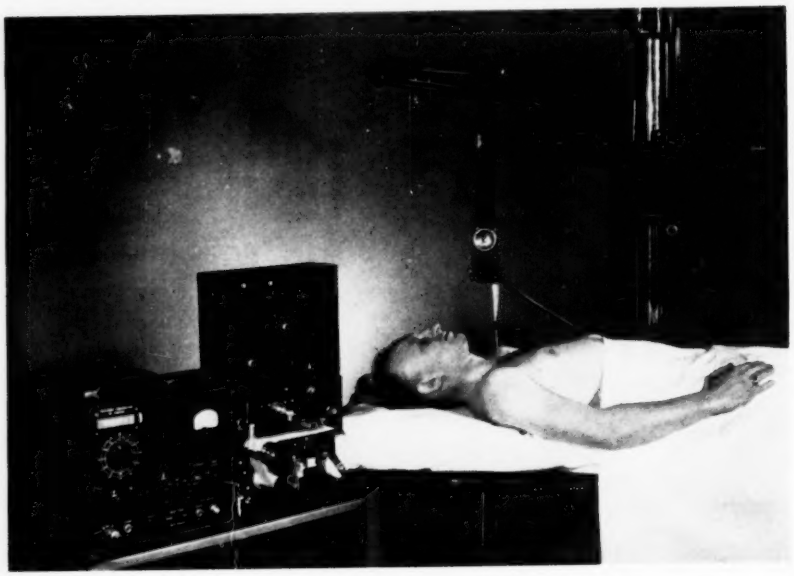

FIG. 1 APPARATUS FOR MEASURING THYROID UPTAKE

the variation of counting rate with distance from the neck followed more closely an inverse linear rather than an inverse square relation and approximated $1/r^{1.3}$. Therefore, an error of $\frac{1}{4}$ to $\frac{1}{2}$ inch in the estimation of the depth of the thyroid within the neck produced an error of only $3\frac{1}{2}$% to 7%, in the counting rate due to activity in the thyroid at the standard nine inch distance.

Radioactivity of the administered doses and in the urine and blood samples was assayed in Marinelli beakers placed around 4 inch lead shielded RCL bismuth walled or tetramethyl lead silver walled glass Geiger counters with sensitivities of 4500 and 479 C/M/μc I^{131}, respectively.

Determination of clearance rates

Since the thyroidal plasma I^{131} clearance is defined as the volume of plasma cleared of I^{131} per minute by the thyroid gland, its evaluation requires the measurement of the uptake of I^{131} and a knowledge of the average plasma concentration over the same time period. Similar considerations obtain for the renal clearance. The first half hour following intravenous injection of the isotope has been selected for the clearance determination. Thus

$$\text{Plasma } I^{131} \text{ clearance}_{\text{thyroid}} = \frac{\text{½ hour thyroid uptake } I^{131}}{\text{average plasma concentration } I^{131} \times 30} \quad (1a)$$

$$\text{Plasma } I^{131} \text{ clearance}_{\text{renal}} = \frac{\text{½ hour renal excretion } I^{131}}{\text{average plasma concentration } I^{131} \times 30} \quad (1b)$$

In the following sections discussion of the measurement of the half hour thyroid uptake and renal excretion is followed by a consideration of the plasma I^{131} concentration determinations.

A. Determination of thyroid uptake and renal excretion

Because of a number of objections to the common use of thigh measurements in correcting for changing extrathyroidal activity in the neck, the following method for determining the thyroid uptake was introduced.

Prior to the injection a background reading is obtained with the counter over the patient's neck. The counting rate at one minute intervals is then recorded for 33 minutes from the time of injection, following which the patient empties his bladder into a bottle free of radioactivity. A five minute delay time in urinary formation and passage is assumed. For example, a urine specimen passed at 35 minutes is considered to represent the 30 minute excretion. Since the accuracy of the renal clearance is only of secondary interest, catheterization is considered unnecessary.

The counting rate obtained over the neck during the first minute or two following injection is a measure of the activity of radioiodine in the blood and extravascular spaces, since, at this time, only a fraction of the radioiodine can have been removed by the thyroid except in cases of marked hyperthyroidism. This activity has been termed the initial extrathyroidal level. In hyperthyroidism the activity obtained during the first minute has been accepted as the initial extrathyroidal level because of the rapid increase in counting rate during the first few minutes in these cases. Any error in the tissue level estimation in the hyperthyroid cases is of little consequence compared to

the total half hour thyroid uptake. In non-hyperthyroid cases the activity of the second minute has been taken as the initial extrathyroidal level since at this time mixing in the blood stream is virtually complete. Although commonly equal to the counting rate of the first minute, the counting rate during the second minute is not infrequently slightly lower than that during the first minute. The reason for this can be appreciated when it is realized that a large number of counts may be recorded during the first few seconds due to the high activity of the concentrated injected material as it first enters the base of the neck through the great veins draining the site of injection.

The counting rate over the neck after any significant period of time is a measure of the activity within the thyroid gland as well as that still remaining in the blood and extrathyroidal tissues of the neck. Thyroid uptake of I^{131} may be calculated as follows:

Let Th = μc I^{131} collected by the thyroid during the first 30 minutes
U = μc I^{131} excreted in the urine during the first 30 minutes
D = dose injected in μc I^{131}
O = observed increase in activity over the neck in μc I^{131} during the first 30 minutes
E = initial extrathyroidal activity in the neck in μc I^{131}, less background value prior to injection.

E thus represents the activity of I^{131} in the extrathyroidal tissues of the neck before any significant removal by the thyroid and kidneys has occurred.

The thyroid uptake must have increased at the end of the measurement period by the sum of the observed increase in the activity of the neck and the fall in the level of activity of extrathyroidal I^{131} in that part of the neck "seen" by the counter. The extrathyroidal changes include the changes in concentration in the blood and extracellular spaces of the thyroid gland itself. At the end of the measuring period, the dose still circulating and in the tissue spaces throughout the body will have decreased by the amount picked up in the thyroid and excreted in the urine since minute amounts that may be concentrated above the general tissue level in other organs are negligible. The fractional fall in the general tissue level may then be expressed as $\frac{Th + U}{D}$. The fall in the extrathyroidal activity of the neck is therefore $\frac{(Th + U)}{D} E$.

Then

$$Th = O + \frac{(Th + U)}{D} E.$$

Solving for Th,

$$Th = \frac{OD + EU}{D - E}. \qquad (2)$$

The value of E is usually less than 10% of the activity of the dose and the urinary excretion usually not more than three times the increase in activity over the neck except in cases of hypothyroidism. It may be noted, then, that any error in urinary collection or measurement produces only a fraction of that error in the determination of the thyroid uptake. In hyperthyroidism, where the thyroid uptake is two to ten times the urinary excretion, the latter may be neglected entirely for practical purposes.

The total 30 minute urinary excretion is measured and the activity assayed as described above.

B. Determination of the average plasma I^{131} concentration during the 30 minute period following injection

Ideally, the plasma concentration should be kept at a constant level during the period of clearance determination. Owing to long continued change of space of iodide dilution as discussed below, maintenance of an unchanging concentration by constant intravenous drip would not only be difficult, but also undesirable, because of the larger dose required.[3] It is, however, equally valid to determine the average plasma concentration of I^{131} over the period of time of measurement of thyroid uptake and renal excretion, even though the concentration is changing.

The plasma concentration of I^{131} at any time following its administration is dependent upon the space into which it has been distributed and the extent to which it has been removed from circulation by the thyroid and kidneys. (Amounts fixed in other organs are negligible.) These two variables are discussed independently below. At the outset, however, a general consideration of the problem is pertinent.

The plasma concentration at any time, t, may be expressed as follows:

$$(\text{Plasma } I^{131} \text{ conc.})_t = D_t \cdot C_t, \qquad (3)$$

where

D_t = dose retained at any time, t,

C_t = $\frac{\text{fraction of dose retained}}{\text{ml plasma}}$ at the same time.

The dose retained at any time is the administered dose less that removed by the thyroid and kidneys up to that time.

The average plasma I^{131} concentration over any time interval following administration of the isotope can then be formulated:

$$(\text{Average plasma } I^{131} \text{ conc.})_{0-T} = \frac{\int_0^T (D_t \cdot C_t)\, dt}{T}. \qquad (4)$$

The integral of the product of the two factors D_t and C_t approximates the product of the integrals of each factor as one or both approach a constant. Thus:

$$\frac{\int_0^T (D_t \cdot C_t)\, dt}{T} \simeq \frac{\int_0^T D_t\, dt}{T} \cdot \frac{\int_0^T C_t\, dt}{T}. \qquad (5)$$

[3] In hyperthyroidism, this procedure would require separate determination of hormonal and inorganic iodide in the plasma, since, by the time complete mixing of I^{131} in the body has occurred (usually three or more hours) a significant amount of I^{131} may have re-entered the circulation in the form of hormonal iodide after having been taken up by the thyroid. Furthermore, for the same reason, determination of thyroid uptake would be in error because of the I^{131} leaving the gland after being incorporated into hormone.

The first integral term on the right hand side of equation 5 is the average dose retained while the second expresses the average plasma concentration in terms of the fraction of the dose retained. The change in C_t with time is therefore dependent only on the change of space of dilution. These two terms have been evaluated separately and the error involved in the approximation considered subsequently.

1. Evaluation of average dose retained. A close approximation to the value of the integrated average dose retained is given by the following:[4]

$$\bar{D}_{0-T} = \frac{\int_0^T D_t\,dt}{T} \cong \frac{D_0\,(1 - e^{-\lambda T})}{\lambda T}, \qquad (6)$$

[4] See Appendix A.

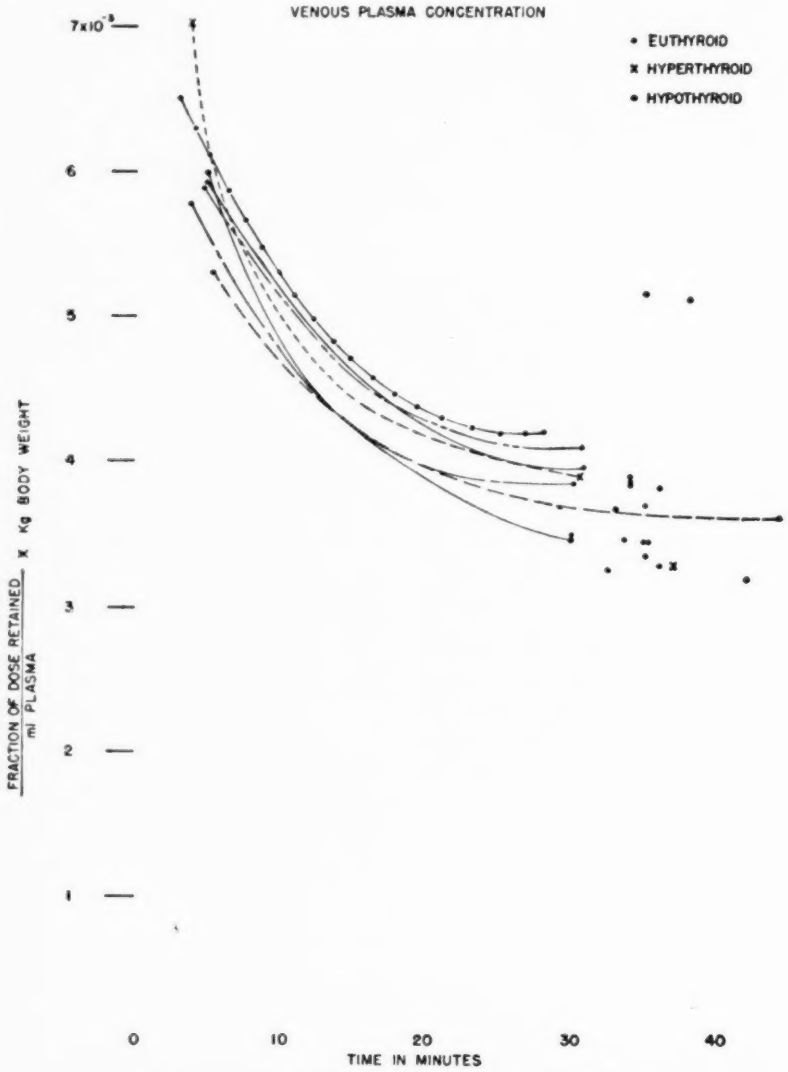

FIG. 2. VENOUS PLASMA I^{131} CONCENTRATION-TIME CURVES

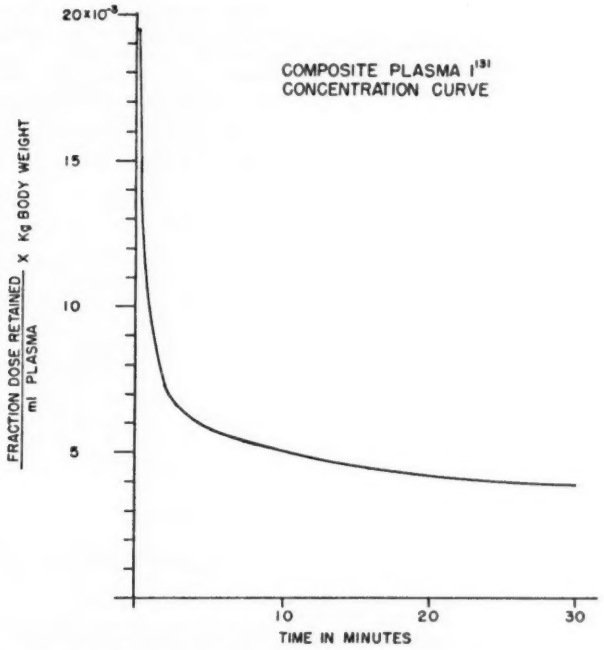

FIG. 3. COMPOSITE PLASMA I^{131} CONCENTRATION-TIME CURVES

Points during the first three minutes were obtained from arterial sample assays.

where $\bar{D}_{O-T}$ = Average dose retained over time interval O–T
D_O = Dose I^{131} administered
T = Time in minutes
λ = Average fraction of the dose removed per unit time. The evaluation of this term is discussed in Appendix A.

While this expression can be evaluated in a number of ways, it may be desirable for the sake of mathematical expediency to approximate the average dose retained by taking the arithmetic mean of the dose administered and the dose retained at the end of the period of measurement.[6]

$$\bar{D}_{O-T} \cong \frac{D_O + D_T}{2} \cong D_O - \tfrac{1}{2}(Th_T + U_T), \qquad (7)$$

where D_T = Dose retained at time T = $D_O - (Th_T + U_T)$
Th_T = Thyroid uptake at time T
U_T = Total renal excretion at time T.

2. Evaluation of average C_t. As shown subsequently $\frac{\int_0^T C_t\,dt}{T}$, when T = 30 minutes, has been found to be quite constantly related to body weight in non-edematous subjects and to have the value of $\frac{5 \times 10^{-3}}{\text{body weight}_{kg}}$ in units of $\frac{\text{fraction of dose retained}}{\text{ml plasma}}$.

3. Derivation of final clearance formulae. Both integrals on the right hand side of equation *5* have now been separately evaluated. Substituting these values in equation *5* for T = 30 minutes gives:

$$(\text{Av. plasma } I^{131} \text{ conc.})_{O-\frac{1}{2}hr} = \bar{D}_{O-\frac{1}{2}hr} \cdot \frac{5 \times 10^{-3}}{\text{body weight}_{kg}}.$$

Substituting back in the original clearance equations *1a* and *1b*:

$$\text{Plasma } I^{131} \text{ clearance}_{thyroid} = \frac{\frac{1}{2} \text{ hour thyroid uptake}}{\bar{D}_{O-\frac{1}{2}hr} \times \frac{5 \times 10^{-3}}{\text{body weight}_{kg}} \times 30}$$

$$= \frac{\frac{1}{2}\text{hour thyroid uptake} \times .20 \times 10^3 \times \text{body weight}_{kg}}{\bar{D}_{O-\frac{1}{2}hr} \times 30}. \qquad (8)$$

$$\text{Plasma } I^{131} \text{ clearance}_{renal} = \frac{\frac{1}{2}\text{hour renal excretion} \times .20 \times 10^3 \times \text{body weight}_{kg}}{\bar{D}_{O-\frac{1}{2}hr} \times 30}. \qquad (9)$$

The average dose retained over the first half hour ($\bar{D}_{O-\frac{1}{2}hr}$) may be calculated from equation *6* or *7*.

[6] See Appendix B.

CONSIDERATIONS OF I^{131} DISTRIBUTION IN THE DETERMINATION OF PLASMA CONCENTRATION

A. Change of plasma I^{131} concentration due to change of space of iodide dilution during the first half hour

During the first half hour following intravenous administration, the I^{131} space of dilution increases rapidly due to entrance of I^{131} into red blood cells, diffusion into extracellular fluid spaces and penetration of the body cellular tissues.

In order to determine the change in concentration of I^{131} due to the change of space of dilution with time, multiple venous blood assays were performed during the 30 minute period following injection of I^{131} in seven subjects including one subject with Graves' disease and one with post-thyroidectomy myxedema. The activity in the plasma and frequently also in the red blood cells was determined at four to five points during this time period after administration of I^{131}. To account for the variation of the space of I^{131} dilution with body size, $C_t \times \text{body weight}_{kg}$ has been plotted as a function of time (Figure 2). The dose retained at each point was calculated by subtracting from the dose administered the amount picked up by the thyroid plus that excreted in the urine up to that time. The thyroid gland is exposed to a concentration of I^{131} approximating that in the arterial blood and it may take two to three minutes for the venous blood to approach the arterial concentration. Therefore the arterial plasma concentration was assayed at half minute intervals during the first three minutes following intravenous administration of the isotope, in order to permit a more accurate extrapolation back to zero time for the concentration of plasma I^{131} to which the thyroid is exposed. The areas under the curves thus obtained were measured with a planimeter. The maximum variation from the mean area was found to be about 3%.

In 17 other subjects, including one patient with Graves' disease, single points at 30–40 minutes were obtained. When all these points were extrapolated along a typical curve to the 30 minute time, the maximum variation of 22 of the 24 30-minute points was less than 11% from the mean. The mean concentration over the first half hour varied less than the concentration at 30 minutes since the relative spread was greater at the end of the time interval and because the curves crossed each other to a certain extent. It is quite possible that the two exceptional points represent technical errors due to low counting rates or slight contamination rather than true deviations.

TABLE I

Three hour iodide space

Patient	3 hour iodide space *% body weight*
85	36.1
29	31.2
86	45.4
82	42.0

Figure 3 shows the composite time-concentration curve, including the results of the arterial plasma assays during the first three minutes. The area under this curve divided by the time interval, 30 minutes, is then the mean concentration over the first half hour expressed as $\frac{\text{fraction dose retained}}{\text{ml plasma}} \times \text{body weight}_{kg}$. Its value was found to be 5.0×10^{-3}. Thus

$$\frac{\int_0^T (C_t \cdot \text{body weight}_{kg})}{T} = 5 \times 10^{-3} \text{ in units of } \frac{\text{fract. dose ret.} \times \text{kg}}{\text{ml plasma}}.$$

Since body weight is a constant for any subject, then

$$\frac{\int_0^T C_t\,dt}{T} = \frac{5 \times 10^{-3}}{\text{body weight}_{kg}} \text{ in units of } \frac{\text{fract. dose ret.}}{\text{ml plasma}}.$$

This is the value of $\frac{\int_0^T C_t\,dt}{T}$ utilized in the clearance calculations described under methods.

B. "Iodide space"

The reciprocal of $\frac{\text{fraction dose retained}}{\text{ml plasma}}$ obviously represents the space of dilution. The reciprocal of any point along the curve in Figure 3 thus represents $\frac{\text{space in ml}}{\text{body weight in kg}}$, or taking a liter of plasma as equivalent to a kilogram, the space, in per cent of the body weight, through which the I^{131} would have had to be uniformly distributed in order to have given the experimentally obtained plasma concentration. In terms of any anatomical space in the body, this has no significance, since the I^{131} is not uniformly distributed between extracellular and intracellular tissues. For example, the plasma/red cell ratio of I^{131} has ranged between 1.6 and 1.8 for all determinations made between three minutes and three hours after injection. However, the "iodide space" may serve as a convenient mathematical expression for purposes of calculation of plasma concentration and gives a rough quantitative idea of the extent of distribution. At 30 minutes, in 22 of the 24 observations the mean and maximum range of the "iodide space" was 26 ± 2.8% of body weight in non-edematous subjects. However, at three hours the variation was much greater, the space ranging from 31 to 45% of the body weight (Table I). The average space over the first half hour was found to be 20% of the body weight.

In a patient with considerable nephrotic edema, the "space" at 30 minutes was 38% of his body weight compared to the normal of 26%. In a patient with cardiac edema the average "space" over the first half hour was 26% of his body weight compared to the normal of 20%. For accurate determinations in cases with significant edema it is therefore necessary to obtain several plasma assays during the first half hour, although the error introduced by using an estimated correction of the normal space does not influence the clearance values sufficiently to confuse the diagnosis when hyperthyroidism is present.

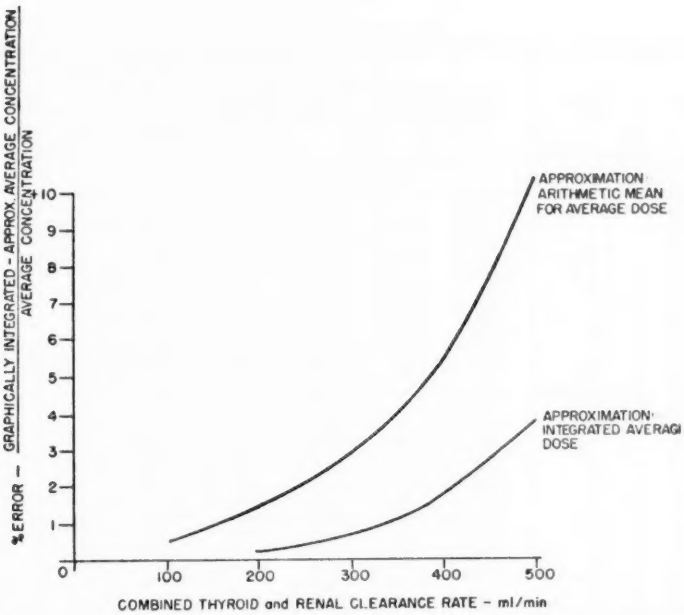
% ERROR — GRAPHICALLY INTEGRATED - APPROX. AVERAGE CONCENTRATION / AVERAGE CONCENTRATION
APPROXIMATION: ARITHMETIC MEAN FOR AVERAGE DOSE
APPROXIMATION: INTEGRATED AVERAGE DOSE
COMBINED THYROID and RENAL CLEARANCE RATE - ml/min

FIG. 4. PER CENT ERROR INVOLVED IN MATHEMATICAL APPROXIMATIONS AT DIFFERENT CLEARANCE RATES

See text

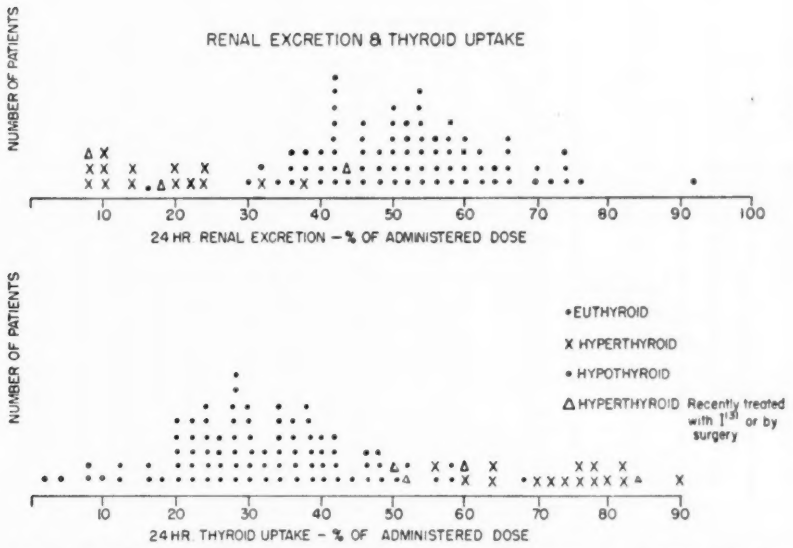
RENAL EXCRETION & THYROID UPTAKE
NUMBER OF PATIENTS
24 HR. RENAL EXCRETION — % OF ADMINISTERED DOSE
•EUTHYROID
X HYPERTHYROID
• HYPOTHYROID
Δ HYPERTHYROID Recently treated with I^{131} or by surgery
24 HR. THYROID UPTAKE - % OF ADMINISTERED DOSE

FIG. 5. FREQUENCY DISTRIBUTION OF 24 HOUR RENAL EXCRETION AND THYROID UPTAKE VALUES EXPRESSED AS PER CENT OF THE ADMINISTERED DOSE

TABLE II

Patient	Age	Sex	Height	W (kg)	Surface area (m^2)	24 hour uptake (% dose)	24 hour excretion (% dose)	Plasma I^{131} clearance (ml/min.)		Plasma I^{131} clearance (ml/min./1.73 m^2)	
								Thyroid	Renal	Thyroid	Renal
Euthyroidism											
1	26	M	6′	73.1	1.93	22.2	73.5	8.0	50.4	7.2	45.2
2		M	5′5″	60.5	1.65	57.4	41.7	29.3	10.6	30.7	11.1
3	35	M	5′9¼″	75.5	1.91	68.0	31.0	44.2	14.3	40.0	12.9
4		M	5′11½″	71.5	1.90	28.4	60.4	15.4	25.0	14.0	22.7
5	39	M	5′11″	66.4	1.83	55.0		29.9	21.2	28.2	20.0
6	26	M	5′9″	77.2	1.92	15.0	76.0	6.3	54.0	5.7	48.7
7	28	M	5′11″	73.0	1.90	33.0	62.2	14.0	33.2	13.4	31.9
8	60	M	5′8″	63.2	1.73	27.2	73.4	8.9	37.4	8.9	37.4
9	58	M	5′11″	69.0	1.96	46.0	50.0	30.3	31.3	26.7	27.6
10	62	M	5′5¼″	59.0	1.63	25.0	46.3	6.3	15.5	6.7	16.4
11	27	M	5′10″	53.0	1.64	30.0	41.8	14.8	44.0	15.6	46.4
12		M	5′3″	70.0	1.72	22.0	54.0	17.5	34.2	17.6	34.3
13		M	5′6″	68.5	1.76	35.0		38.4	28.1	37.8	27.6
14	31	M	5′10½″	73.0	1.90	13.0		11.6	44.0	10.5	40.0
15	25	M	5′11″	70.0	1.87	46.0	54.0	23.3	44.5	21.5	41.2
16	32	M	5′8″	54.0	1.61		52.0	37.9	28.6	40.8	30.8
17	29	M	5′9″	75.0	1.89	23.0	52.5	17.2	45.0	15.7	41.1
18	37	M	6′2″	83.4	2.08	48.8	51.2	20.0	24.1	16.6	20.0
19	32	M	5′8½″	65.0	1.77	48.0	34.0	18.8	20.8	18.3	20.3
20	61	M	5′9″	64.5	1.77			22.6	23.2	22.0	22.6
21	61	M	5′9″	63.6	1.76	12.0	66.0	15.3	33.1	15.1	37.6
22	58	M	5′7½″	56.3	1.63	50.5		33.2	27.0	35.2	28.7
23	48	M	5′6″	49.0	1.53	42.0	50.0	27.9	21.0	31.5	23.8
24	42	M	5′8½″	79.5	1.88	41.0	60.0	42.0	54.0	38.7	49.7
25	28	M	6′1″	75.0	1.96	47.0	53.0	39.8	32.2	35.2	28.4
26	24	M	5′3″	61.6	1.62	25.0	53.0	19.1	51.2	20.4	54.6
27		M	5′7″	79.0	1.89			15.8	45.0	14.4	41.2
28	44	M	5′4½″	43.2	1.43	19.0	45.0	5.7	22.8	6.9	27.7
29	39	F	5′6½″	63.8	1.71	31.4	58.3	15.2	29.8	15.4	30.2
30	51	M	6′0″	74.5	1.94	34.2	62.5	22.3	38.7	19.9	34.5
31	54	M	6′1½″	75.5	1.99	39.0	55.0	25.7	44.2	22.3	38.4
32	42	M	5′3½″	76.0	1.79	28.0	57.0	27.4	52.2	26.5	50.5
33	45	M	5′6½″	61.2	1.69	48.0	42.0	25.7	20.8	26.3	21.3
34	29	M	5′7½″	61.2	1.71			30.0	34.3	30.3	34.7
35	33	M	5′9¾″	88.3	2.05	38.6	55.0	25.4	52.0	21.4	43.9
36	46	M	5′11¾″	95.5	2.14	37.0	58.5	18.1	48.7	14.7	39.4
37	28	M	5′10¾″	79.0	1.96	42.0	63.0	28.4	53.0	25.1	46.8
38	29	M	5′7″	80.0	1.90	22.0	50.0	7.8	31.2	7.1	28.4
39		M	5′8½″	63.3	1.74	27.0	63.0	9.8	53.0	9.7	52.0
40	23	M	5′7″	69.5	1.79	38.0		29.8	41.5	28.8	40.0
41	62	M	5′9½″	80.5	1.95	25.0	36.8	12.1	57.2	10.7	50.7
42	55	M	5′7½″	54.5	1.62	30.0	53.0	20.5	30.9	21.9	33.0
43	64	M	5′1½″	74.5	1.74	30.0		12.9	20.1	12.8	20.0
44	34	M	6′0″	89.0	2.10	28.0	66.0	8.3	38.2	6.8	31.4
45	39	M	5′8″	84.0	1.96			7.7	57.1	6.8	50.5
46	26	M	5′11¾″	88.5	2.09	38.0	49.0	31.0	48.6	25.6	40.2
47	52	M	5′5½″	85.5	1.92	21.0	47.2	12.3	28.8	11.1	25.9
48	64	M	5′4″	57.3	1.59	22.0	60.0	13.5	42.5	14.7	46.2
49	40	M	5′8″	59.0	1.67	19.0		7.1	50.1	7.4	52.0
50	34	M	5′11½″	86.3	2.05	25.0	48.0	9.5	69.0	8.0	58.0
51	54	M	5′5½″	65.8	1.73	44.3	46.8	35.3	49.0	35.3	49.0
52	39	M	6′0″	86.3	2.07			9.2	49.1	7.7	41.0
53	27	M	5′8″	78.0	1.90	40.0	48.0	21.8	61.6	19.8	56.2
54	55	M	5′8″	60.5	1.82	36.0	50.0	17.4	21.6	16.5	20.6
55	30	M	5′5″	84.0	1.90	37.0	40.0	21.8	50.5	19.9	46.0
56	32	F	5′3½″	62.6	1.65	43.5	46.7	39.2	34.8	41.0	36.6
57	58	M	5′6″	81.5	1.87	28.0	57.0	8.1	29.5	7.5	27.3
58	30	M	5′8½″	75.9	1.89	37.5	53.0	21.0	48.2	19.2	44.3

TABLE II—*Continued*

Patient	Age	Sex	Height	W (*kg*)	Surface area (m^2)	24 hour uptake (*% dose*)	24 hour excretion (*% dose*)	Plasma I^{131} clearance (*ml/min.*)		Plasma I^{131} clearance (*ml/min./1.73 m²*)	
								Thyroid	Renal	Thyroid	Renal
Euthyroidism—*Continued*											
59	52	M	5′11″	68.5	1.86	20.0	59.0	15.8	18.5	14.7	17.2
60	17	F	5′2½″	57.6	1.57	42.5	45.4	17.0	21.2	18.7	23.4
61	45	M	5′11″	72.5	1.91	20.0	67.0	11.6	39.2	10.5	35.4
62	28	M	5′11″	88.0	2.07	23.0	50.0	8.8	35.2	7.4	29.4
63	41	M	5′6½″	75.0	1.84	25.0	60.0	24.0	42.7	22.5	40.1
64	45	F	5′7″	60.0	1.69	28.0	55.0	6.0	28.2	6.1	28.9
65	40	F	5′4″	64.5	1.68	33.9	57.7	20.6	34.8	21.2	35.8
66	44	M	5′9″	91.4	2.05	19.0	73.0	11.9	61.2	10.1	51.7
67	55	M	5′10½″	81.8	1.99	51.0		23.7	20.4	20.6	17.7
68	38	M	5′11″	66.0	1.83	34.0		21.8	33.6	20.6	31.7
69	28	M	5′11½″	89.0	2.09	37.6	42.0	24.0	38.2	19.8	31.5
70	41	F	5′5½″	79.6	1.86	57.6	42.0	40.4	51.0	37.6	47.5
71	55	M	5′7½″	59.0	1.68	35.0	65.0	3.6	25.0	3.7	25.8
72	59	M	5′6¾″	82.8	1.93	29.3	44.0	13.6	33.9	12.2	30.4
73	35	F	5′7½″	82.3	1.94	31.0		8.0	28.0	7.2	25.0
74	52	F	5′2″	45.4	1.42	29.0	56.0	8.6	15.4	10.5	18.7
75	28	M	5′10″	79.6	1.96	32.0	61.5	15.2	54.2	13.4	47.8
76	37	M	5′7″	64.5	1.73	30.0	53.0	16.8	51.6	16.8	51.6
77	32	F	5′5″	59.8	1.69	16.2	64.2	5.1	47.5	5.2	48.5
78	24	F	5′7½″	71.0	1.83	22.0	70.5	8.5	54.6	8.0	51.7
79	38	M	5′9″	91.0	2.04	23.6	58.7	14.6	52.0	12.4	46.1
80	26	M	5″9″	81.3	1.95			10.8	46.0	9.6	40.8
81	63	M	5′1¼″	48.2	1.44	27.2	40.4	10.0	16.1	12.0	19.3
82	56	M	5′4½″	66.0	1.70	36.9	54.3	19.1	30.4	19.4	30.9
83	60	M	5′9″	70.4	1.83	39.5	58.0	11.3	51.4	10.7	48.5
84	48	M	5′5½″	60.4	1.64	27.4	41.8	8.4	28.0	8.8	29.5
85	56	M	5′7″	47.6	1.54	25.5	55.2	8.1	28.0	9.1	31.5
86	31	M	5′2″	57.6	1.56	39.6	40.3	21.5	44.2	23.8	49.0
87	34	M	6′1″	69.5	1.91	35.1	54.2	21.2	30.2	19.2	27.3
Hyperthyroidism											
1	45	M	5′4½″	66.0	1.67	63.0	13.9	94.5	38.2	97.5	39.4
2	63	M	6′2″	51.4	1.70	82.0	24.0	73.5	19.0	75.0	19.3
3	33	M	5′9″	61.3	1.79	73.0	7.0	371.0	24.5	358.0	23.7
4	54	M	5′8″	69.0	1.81	70.0	20.0	345.0	24.8	330.0	23.7
5	34	M	5′10″	59.0	1.72	78.5	20.0	210.0	35.4	211.0	35.6
6	63	M	5′7½″	70.5	1.81	56.0	32.0	77.5		74.5	
7	55	M	5′5½″	64.6	1.71	64.0	38.0	82.0		83.0	
8	32	M	5′8½″	53.5	1.63	78.0	23.0	257.0	32.1	273.0	34.1
9	34	M	5′10″	60.0	1.73	71.0	14.0	115.0	15.6	115.0	15.6
10	54	M	5′4″	66.0	1.69			82.5	43.5	84.5	44.5
11	61	M	5′8″	60.5	1.70	75.8	10.4	74.0	5.1	75.5	5.2
12	37	M	5′9″	63.0	1.75	81.3	8.0	360.0	22.0	356.0	21.7
13	34	M	5′6½″	56.8	1.63	77.0	10.3	387.0	34.9	410.0	37.0
14	61	M	5′5″	52.0	1.54	80.0	10.0	206.0	23.2	232.0	25.0
15	45	M	5′11″	69.4	1.88	61.0	24.0	103.0	39.0	92.0	36.0
16	62	M	5′7″	67.5	1.77	90.0	11.0	523.0	52.0	512.0	51.0
17	25	F	5′8½″	81.8	1.95	81.0		229.0	60.0	203.0	53.3
18	63	F	5′4″	51.0	1.52	84.8	9.7	188.0	17.2	214.0	19.6
Hypothyroidism											
1	47	F	5′1½″	70.0	1.69	3.0	73.0	1.2	39.2	1.2	40.0
2	23	M	5′10″	65.0	1.80	10.1	92.0	1.2	38.4	1.5	36.9
3	73	M	5′6¼″	79.5	1.89	7.5	32.0	0	15.4	0	14.1
4		M	5′8″	66.0	1.77	7.7		4.2	9.3	4.1	9.1
5	55	M	5′5½″	64.5	1.70	2.9	70.0	3.2	33.8	3.2	34.0

It has been noted by others (17) that the "iodide space" in thyrotoxic patients may be increased over that found in normal subjects. This has not been observed in the few cases which we have studied. However, when the blood volume is markedly increased and edema associated with decompensated heart disease as a consequence of the toxic state is present, such an increase in the "iodide space" is to be expected. None of our hyperthyroid patients showed such changes.

C. Evaluation of errors introduced by approximations

In the section on Methods, several approximations were made in the mathematical treatment of the changes in concentration with time. The validity of the approximations made in equations *5*, *6*, and *7* under the conditions of the present study has been tested by graphic integration.

From the observed values of "iodide space" determined at minute intervals from the curve in Figure 3, calculated plasma concentration-time curves were obtained for combined thyroid and renal clearance rates of 100–500 ml/min. The mean concentrations over the first half hour determined by integrating the areas under these curves were then compared with the mean concentrations calculated from equation *5*. Both the approximate integrated dose retained (equation *6*) and the arithmetic mean dose retained (equation *7*) were employed in calculating the average concentrations from equation *5*. The per cent difference between the graphically integrated and the calculated average concentrations (*i.e.*, the error involved in the approximations under discussion) was then plotted as a function of the combined thyroid and renal clearance rates (Figure 4). At the highest combined clearance rate in euthyroidism (100 ml/min., see Table III) the error is negligible (less than 1%), if either the approximate integrated or the arithmetic mean dose retained is used to calculate the average concentration. At the second highest yet observed combined clearance rate (450 ml/min.) the error involved when the approximate integrated dose is used is only 2½–3% and is still less than 10% even when the arithmetic mean dose is accepted as the average dose retained.

For more accurate evaluation of the clearance rates in hyperthyroidism, correction for the approximations involved in the calculations of the average concentration may be obtained from Figure 4. The only approximation which is not corrected for is in using the average "iodide space" over the first half hour as 20% of body weight, but, as noted above, this has been observed to be strikingly constant in non-edematous subjects with a maximum probable range of ±10% from the mean.

RESULTS

A. 24 hour thyroid uptake and renal excretion values

In Table II are presented the clearance data and the 24 hour thyroid and urinary I^{131} accumulation values in 87 euthyroid, 18 untreated hyperthyroid and five hypothyroid subjects, four of whom were frankly myxedematous. Studies in five cases of hyperthyroidism recently treated with therapeutic doses of I^{131} or by subtotal resection, with only partial clinical success, are also included. All of these five patients shortly thereafter relapsed into full blown toxicity.

Figure 5 shows the frequency distribution of the 24 hour thyroid uptake and renal excretion values expressed as per cent of the administered dose. The range and average values for each clinical group are presented in Table III. While there is fairly good diagnostic separation of the clinical groups there remains a definite overlap with either test. The chart on renal excretion excludes 16 cases in which the urine collection was known to be incomplete. Since in any particular case, the reliability of a urine collection may be questioned, this measurement is even less suitable as a diagnostic test than might be inferred from the data pictured in Figure 5.

A thyroid uptake of 50% or more at the end of 24 hours has heretofore been accepted in this clinic and elsewhere as indicative of hyperthyroidism. Seven cases of euthyroidism are noted to fall in this range.

Excluding the cases in which the urine collection was known to be incomplete, an average of 87% of the administered dose was accounted for in the thyroid and urine at the end of 24 hours in the euthyroid subjects.

B. Half hour thyroid uptake curves

In contrast to the overlap between euthyroid and hyperthyroid cases in the 24 hour thyroid uptake measurements, the thyroid uptake curves during the first half hour reveal that a better differentiation is possible at this time (Figure 6).

TABLE III

Range and mean values for 24 hour thyroid uptake and renal excretion

Clinical state	Thyroid uptake (% of dose)		Renal excretion (% of dose)	
	Range	Mean	Range	Mean
Hyperthyroid	56.0–90.0	70.1	7.0–38.0	18.1
Euthyroid	12.0–68.0	33.0	31.0–76.0	53.7
Hypothyroid	2.9–10.1	6.2	32.0–92.0	66.8

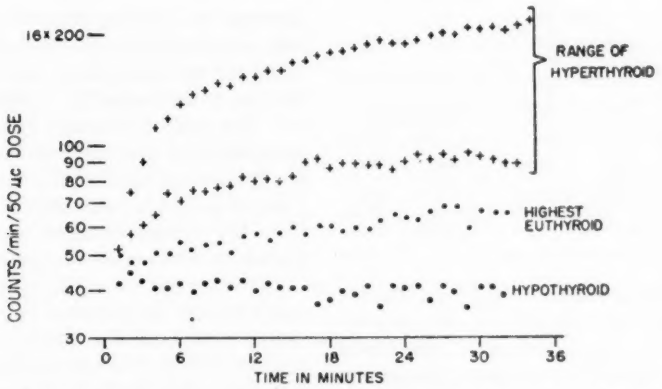

FIG. 6. COUNTING RATE OVER THE NECK DURING THE FIRST HALF HOUR FOLLOWING INTRAVENOUS ADMINISTRATION OF I^{131} TO PATIENTS IN DIFFERENT CLINICAL STATES

C. Thyroid and renal clearance values

The frequency distributions of the thyroid and renal clearances are shown in Figure 7. The clearance rates have been corrected to 1.73 m^2 surface area to facilitate comparison between different individuals. The range and mean values for each clinical group are presented in Table IV. There appears to be no real difference in renal clearance rates among the various clinical groups. It may be assumed that the iodide ion is readily filtered through the glomerulus. The clearance values are then consistent with the interpretation that the iodide is reabsorbed by the tubules. That the variations in renal clearance among normal individuals may be related to tubular mechanisms regulating the excretion of other electrolytes, particularly chloride, must be considered. This has not been investigated. It may be noted, however, that only the two lowest points obtained were associated with known renal disease.

In contrast to the distribution of the renal clearance values the thyroid clearances show a clearcut

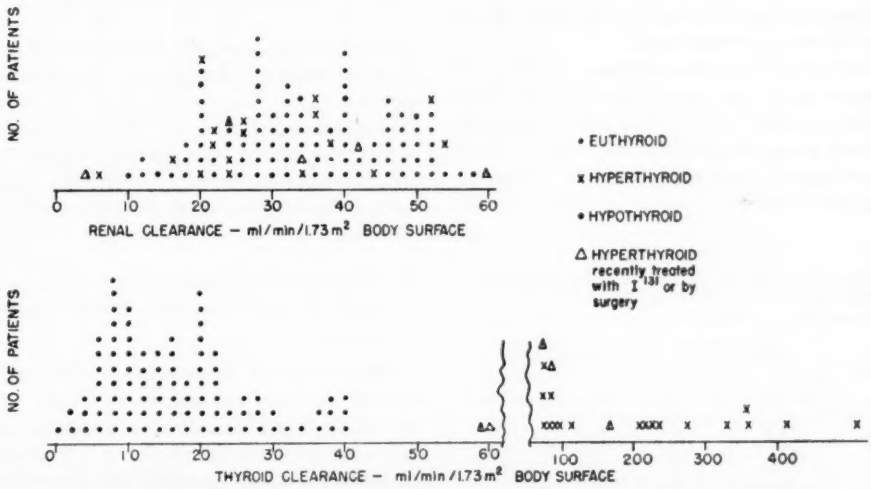

FIG. 7. FREQUENCY DISTRIBUTION OF RENAL AND THYROIDAL PLASMA I^{131} CLEARANCE RATES

TABLE IV

Range and mean values for thyroid and renal clearances

Clinical state	Thyroid clearance (ml/min./1.73 m²)		Renal clearance (ml/min./1.73 m²)	
	Range	Mean	Range	Mean
Hyperthyroid	74.5–512	210.5	5.2–53.3	30.3
Euthyroid	3.7– 41.0	17.7	11.1–58.0	35.6
Hypothyroid	0.0– 4.1	2.0	9.1–40.0	26.8

separation between euthyroid and hyperthyroid cases, with a less well-defined delineation between the euthyroid and hypothyroid cases. The lowest clearance rate observed in untreated hyperthyroidism was almost twice the highest euthyroid clearance. Even in the patients who were in partial clinical remission as a result of therapy and who shortly thereafter showed complete relapse, the clearance rates were significantly beyond the euthyroid range. In general the hyperthyroid patients with markedly enlarged glands showed the highest clearance rates.

The number of euthyroid cases is large enough to define reliably the expected range of normal. It is unlikely that a significant fraction of euthyroid clearance values will be found beyond this range. Owing to the small number of observations in hyperthyroid cases, it is possible that clearances below the present limit may occur. However, because of the marked separation between the clinical groups, it is unlikely that any significant degree of overlap will be encountered.

In order to evaluate the constancy of the thyroid clearance rate in the same individual, the values obtained from the half hour study were compared, in a small group of euthyroid subjects, with those obtained between one half hour and three hours following the administration of I^{131} (Table V). The clearance rates during the one-half hour to three hour period were calculated from the thyroid uptake values and the actual plasma concentrations during this time as determined by repeated plasma analyses. The relative constancy of the thyroid clearances observed here confirms the results of Myant, Pochin, and Goldie (17) in studies done over a longer period in two subjects. In one patient (No. 13) thyroid clearance determinations performed six weeks apart were almost identical.

To further test the validity of the area measurements and the mathematical approximations previously considered, the clearance rates during the first and second 15 minute periods were compared with each other and with the overall 30 minute clearance rate in seven subjects (Table VI). The clearance rates during each 15 minute period were obtained in the same manner as the 30 minute clearance rates by determining the areas under each half of the curve in Figure 3. The average "iodide spaces" during the first and second 15 minute periods were found to be 16.5% and 24.8% of body weight respectively. In general, the agreement of the values is within the error of the measurements.

TABLE V

Comparison of clearance values determined during the first half hour and during one-half to three hours

Patient	Thyroid clearance (ml/min./1.73 m²)		Renal clearance (ml/min./1.73 m²)	
	0–½ hour	½–3 hours	0–½ hour	½–3 hours
85	9.1	8.5	31.5	27.3
29	15.4	14.2	30.2	22.0
86	23.8	19.0	49.0	40.5
82	19.4	14.5	30.9	25.2

TABLE VI

Comparison of clearance values determined over different periods during first half hour

Patient	Thyroid clearance (ml/min./1.73 m²)		
	0–½ hour	½–½ hour	0–½ hour
86	23.0	24.2	23.8
82	19.9	18.2	19.4
24	37.4	38.8	38.7
25	35.9	29.7	35.2
81	12.7	10.9	12.0
23	24.2	41.0	31.5
13—9/28/50			37.8
11/9/50	32.7	36.7	36.2

D. Correlation of thyroid/renal clearance ratios with 24 hour thyroid uptake/renal excretion ratios

Figure 8 shows the correlation of the 24 hour thyroid uptake/renal excretion ratios plotted against the thyroid/renal clearance ratios. In this figure are included the results of 118 studies in 89 subjects. Cases in which urinary collections were known to be incomplete have been excluded.

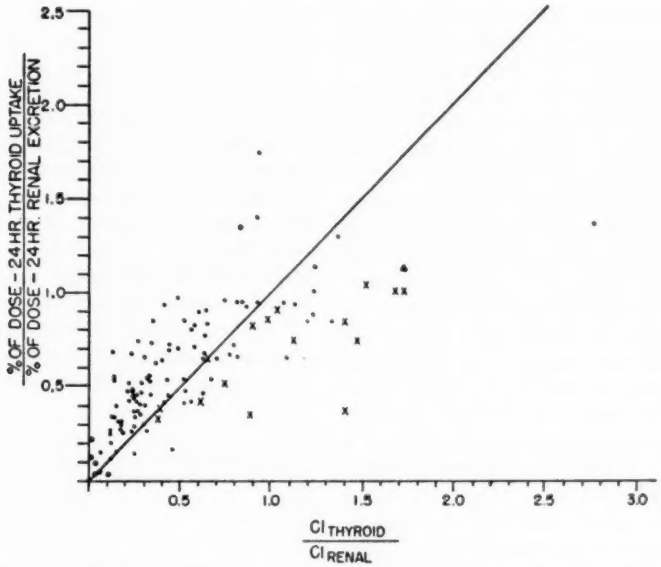

FIG. 8. CORRELATION BETWEEN 24 HOUR THYROID UPTAKE/RENAL EXCRETION RATIOS AND THYROID/RENAL CLEARANCE RATIOS

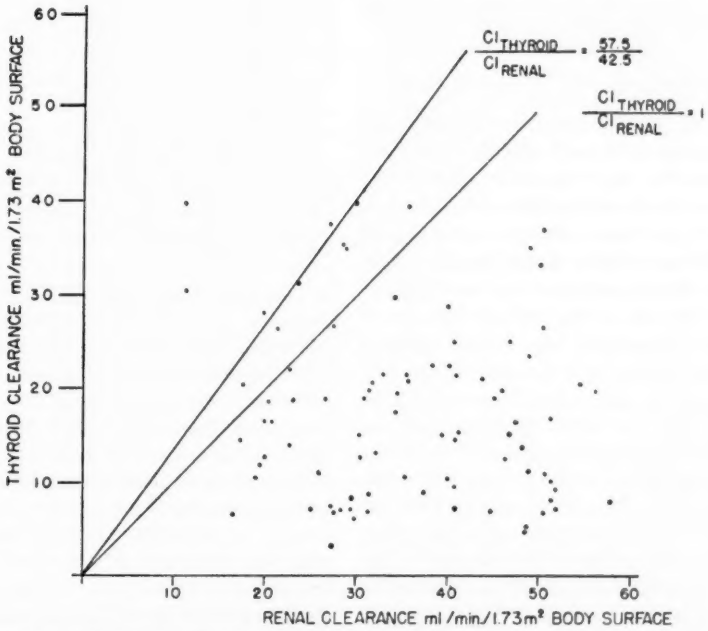

FIG. 9. CORRELATION BETWEEN THYROID CLEARANCE AND RENAL CLEARANCE RATES IN EUTHYROID CASES

In the hyperthyroid cases both ratios were divided by 10 in order to bring the points onto the same graph. While the correlation is good (4-fold $r = 0.73$), it should be noted that the majority of the euthyroid cases fall somewhat to the left of the 45° line through the origin. Since the evidence favors a constant thyroid clearance, this indicates that the average 24 hour renal clearance is slightly lower than that obtained during the test period. This is probably related to temporary alterations in renal function produced by changes in position and activity. In contrast, the points in all but one of the hyperthyroid cases fall to the right of the 45° line. Considering the increased rate of turnover of iodine in the hyperfunctioning gland, the finding of a 24 hour thyroid uptake/renal excretion ratio lower than the respective clearance ratio is not unexpected. Significant amounts of radioiodine may be released in an organically bound form within the 24 hour period. This results in an apparently lower thyroid uptake. Furthermore, some of the iodine previously accumulated by the thyroid may be excreted directly in organically bound form or as iodide resulting from metabolic destruction of the organic iodine compounds, thus leading to falsely high urinary excretion values.

E. Effect of renal clearance on thyroid uptake

To determine the effect of the renal factor on the thyroid uptake, the thyroid clearance rates were plotted against the renal clearance rates of the euthyroid cases as shown in Figure 9. There is no significant correlation between them (4-fold $r = 0.1$). The probability of the thyroid uptake exceeding the renal excretion at any time is represented by the per cent of points lying to the left of the 45° line intercepting the origin. Eleven cases or 12.6% fell into this position. This value then represents the expected frequency of a 24 hour thyroid uptake of 43.5% or greater, since at the end of 24 hours, an average of 87% of the dose was accounted for in the thyroid and urine. Thirteen cases or 16.2% of the actual 24 hour values fell in this range. Similarly the expected frequency of a thyroid uptake of 50% or more of the dose is indicated by the per cent of points to the left of the line whose slope is 50/37 or 57.5/42.5. Six points or 7% fell on or to the left of this line, corresponding exactly to the number of cases found to have a thyroid uptake of 50% or greater at the end of 24 hours.

DISCUSSION

Inquiry into the application of clearance methods to the study of thyroid function was initiated by the British group of workers, whose reports (17–20) represent the only major contributions in this field. These investigators calculated the clearance rates from data obtained by multiple assays of thyroid activity and plasma concentration over a period of several hours following administration of I^{131}. The clearance values of thyrotoxic patients observed by them showed a progressive fall with time to zero levels in five to seven hours. As may be seen from Table VII, which gives for different clearance rates the time required for removal by the thyroid and kidneys of half the dose, at high clearance rates virtually the entire dose may be removed within two hours or less. It should be also noted that in thyrotoxic patients, clearance values obtained more than an hour or two after administration of the dose may represent significant underestimations since at this time appreciable amounts of radioiodine are being released from the gland as hormone, compared to the reduced amounts entering the gland. These points emphasize the necessity for performing the measurements as early as possible when dealing with very active glands. Further, it is desirable to perform the en-

TABLE VII

Calculated removal of radioiodine based on an estimated constant 40% space in a 70 kg man

Combined clearance rates (*ml/min.*)	Half time for removal (*hours*)	% removed at 24 hours
10	32	40.3
20	16	64.4
30	10.7	78.8
40	8.0	87.3
50	6.4	92.4
60	5.3	95.6
70	4.6	97.3
80	4.0	98.4
90	3.6	99.0
100	3.2	99.4
200	1.6	99.99
300	1.1	
400	0.8	

Since, during the early period of time following the administration of the dose, the space is considerably smaller than the estimated 40%, the half time for removal will be appreciably less than the times given in the table above for the higher clearance rates; *e.g.*, the half time for removal at a combined clearance rate of 400 ml/min. is less than 20 minutes.

tire determination at a single sitting in order to avoid the inevitable errors inherent in repositioning the counter.

The thyroid clearance values obtained by Myant, Pochin and Goldie (17) in eight control subjects showed a range of 8 to 38 ml/min. with a mean value of 16 ml/min. Clearance rates in untreated thyrotoxic patients ranged from 198 to 1,390 ml/min. with a mean value of 486 ml/min. The renal clearance rates ranged from 11 to 46 ml/min. with a mean of 27 ml/min. No significant difference in the renal clearance rates between control and thyrotoxic subjects was observed. The only other figures on I^{131} clearance that have come to our attention are those given by Keating and associates (21) in eight cases and those of Stanley (22) in three cases. In the series of Keating and coworkers three euthyroid patients with nodular goiter had thyroid clearances varying from 2 to 13 ml/min., while five patients having exophthalmic goiter had clearances ranging from 20 to 228 ml/min. Our results are more in accord with those of the British workers (17) and show almost the identical range of normal values. This latter group has considered the influence of the extraction ratio on the clearance rate and has pointed out that not only must blood flow be considerably increased over the normal rate of 3½ to 6 ml per gram of tissue per minute (23) but also that extraction must be very nearly complete in markedly overactive glands.

The present study, in agreement with many others, reveals that approximately 9/10 of an administered dose can be accounted for within the thyroid gland and the urine at the end of 24 hours, when thyroid and kidneys are functioning normally. Measurements taken 24 hours or longer after administration of a tracer dose, therefore, do not give an estimate of the absolute level of thyroid function but only express the relationship between the thyroid and kidneys in their ability to clear the blood of iodide. From Table VII, it may be seen that at high renal clearance rates almost all of the dose is removed at 24 hours even in the absence of functioning thyroid tissue.

Since the thyroid clearance remains fairly constant, at least for periods of several hours to a day, the rate of thyroid uptake is necessarily influenced by the rate of renal clearance and the changes with time in the space of I^{131} distribution within the body. Measurements made on the thyroid alone do not take into account either of these factors. Determination of the changes in plasma concentration of I^{131} alone does not enable one to distinguish between removal by the thyroid and loss in the urine, nor does it permit evaluation of the changes of I^{131} space. Measurements of the rate of renal excretion, such as described by Keating, Power, Berkson, and Haines (12) eliminated the renal factor in the evaluation of thyroid function, but did not account for the changes in the space of I^{131} dilution with time as was well appreciated by the original workers.

All of these tests have the additional disadvantage of requiring multiple assays of one sort or another. A single thyroid uptake measurement at an early time, in a sense, gives an approximation to the rate of iodide accumulation and serves to give better diagnostic differentiation than 24 hour studies. For example even the half hour uptake curves alone shown in Figure 6 allow for a well marked separation between the euthyroid and hyperthyroid cases, although overlapping between euthyroid and hypothyroid cases was observed. Similarly, in a recent report (24) on a limited number of patients, multiple measurements of thyroid activity during the first hour following intravenous administration of the isotope permitted a good diagnostic differentiation between euthyroidism and hyperthyroidism.

Stanley (22) has shown that at low serum inorganic iodide levels the rate of accumulation of radioiodine is independent of the serum iodide concentration. In his cases, the I^{131} accumulation was depressed only when high serum levels were produced by administration of iodine. This evidence suggests that, in the untreated gland, it is the clearance rate and not the iodide accumulation rate which reflects the level of thyroid function.

The rate of stable iodide (I^{127}) accumulation by the thyroid may be readily calculated as the product of the I^{131} clearance rate and the plasma concentration of inorganic iodide (I^{127}). It has been shown by Taurog and Chaikoff (25) that, as early as 15 minutes after the injection of I^{131}, 95% of the radioactivity present in the thyroid is organically bound. Radioiodine administered to patients with Graves' disease is rapidly released into the blood stream as thyroxine (26). Radioiodine is also identifiable in the protein precipitable

fraction of the serum of euthyroid subjects as early as a few hours following its administration (11). If all the iodide leaving the gland is in hormonal form and if the total iodine content of the gland is not changed significantly, then the rate of I^{127} accumulation by the thyroid is equal to the rate of hormone release expressed in terms of iodine content. Assuming a serum inorganic iodide concentration of approximately 1 μg% (22), then at the mean euthyroid clearance rate of 17.7 ml/min., the average daily hormone formation and release would amount to 255 μg iodine. This is equivalent to 0.39 mg thyroxine or 127 mg USP thyroid extract. While these figures are only approximations, they agree well with the values of Stanley (22) as regards I^{127} accumulation and with Greer's (27) estimates of thyroid extract equivalent produced daily. They are also close to the daily requirements of myxedematous patients.

Where significant amounts of iodine have been recently ingested or when drugs affecting thyroid function have been administered, these speculations lead to invalid conclusions. As an example it may be noted that in hyperthyroid patients under the influence of such drugs as propylthiouracil and tapazole, the clearance rates may remain considerably elevated (28), indicating continued ability of the thyroid temporarily to accumulate radioiodine. Since hormone formation is blocked by the drug, however, the iodide is rapidly released from the gland as such, and in this circumstance there is no direct relationship between the rate of iodine accumulation and the rate of hormone formation. Ideally, on theoretical grounds, the rate of hormone formation and utilization would be most likely to reflect the clinical state of thyroid function. No publications satisfactorily dealing with the direct solution of this problem have yet appeared. Some recent studies (29–31), reporting the level of organically bound I^{131} in the serum at various times following the administration of the isotope, have failed to take into consideration differences in the stable iodine content of the thyroids of different individuals and the time required to achieve constant specific activity within the body. In any event, this measurement alone does not give more than a first approximation to the rate of hormone formation.

SUMMARY AND CONCLUSIONS

1. The thyroidal and renal plasma I^{131} clearance rates may be obtained in non-edematous individuals with good approximation by a method which is suitable for routine diagnostic use and which does not require the analysis of blood samples. The method is based on an observed relationship of relative constancy between the body weight and the space of I^{131} dilution during the first half hour following intravenous administration of the isotope. The clearance rates are readily determined in a single 35 minute sitting from the assay of radioactivity in the neck and in a single urine specimen.

2. The clearance rates and the 24 hour thyroid uptake and renal excretion values for 87 euthyroid, 18 untreated hyperthyroid, five treated hyperthyroid and five hypothyroid patients are presented. Overlap between euthyroid and hyperthyroid 24 hour values was obtained. The lowest thyroidal clearance rate in hyperthyroidism was almost twice the highest rate in euthyroidism. From analysis of the clearance rates it is to be expected that approximately 7% of the 24 hour values of normal subjects will fall into the hyperthyroid range.

3. In euthyroidism and hyperthyroidism the clearance rates are such that at the end of 24 hours approximately 90% or more of the dose has been removed by the thyroid and kidneys. The fraction of the dose of radioiodine concentrated in the thyroid, especially at this time, is modified by a number of extraneous factors, chief of which is the rate of renal clearance. Since the thyroid clearance rate is independent of these factors it is the most direct and reliable index of the iodine accumulating function of the thyroid gland.

4. The relationship of the thyroidal I^{131} clearance rate to the rate of thyroid hormone formation is discussed.

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APPENDIX A

If the space of dilution and the rate of removal by the thyroid and kidneys remained constant the dose retained would decrease exponentially with time according to the formula:

$$D_t = D_0 e^{-\lambda t}, \qquad (a)$$

where

D_t = dose retained at any time, t
D_0 = the administered dose
λ = the constant fraction of the dose removed per unit time.

The relative constancy of the clearance rates over periods up to three hours has been verified in the present study confirming the findings of Myant, Pochin, and Goldie (17) in normals.

The average dose retained over any time interval could then be expressed as

$$\bar{D}_{0-T} = \frac{\int_0^T D_0 e^{-\lambda t}\, dt}{T}, \qquad (b)$$

where $\bar{D}_{0-T}$ = average dose retained over time interval O − T. λ may be obtained from equation (*a*) as follows:

$$\lambda = \frac{-\ln \frac{D_T}{D_0}}{T}, \qquad (c)$$

where D_T = dose retained at time T.

Since the space of dilution is changing during the first half hour following injection, λ is a variable and these relationships do not hold as absolute equalities. However, the change in λ is such that for values of λT which are small compared to 1, the following approximation is valid. (See Figure 4.) Integrating (*b*) between the designated limits,

$$\bar{D}_{0-T} \cong \frac{D_0(1 - e^{-\lambda T})}{\lambda T}, \qquad (d)$$

where

$\bar{D}_{0-T}$ = approximated integrated dose retained
λ = average value obtained from (*c*).

APPENDIX B

That this approximation is valid for small values of λT can be shown as follows:

$$\bar{D}_{0-T} = \frac{\int_0^T D_0 e^{-\lambda t}\, dt}{T} = \frac{D_0(1 - e^{-T\lambda})}{\lambda T}.$$

Expanding $(1 - e^{-\lambda T})$

$$\bar{D}_{0-T} = \frac{D_0}{\lambda T}\left\{1 - \left(1 - \lambda T + \frac{\lambda^2 T^2}{2!} - \frac{\lambda^3 T^3}{3!} + \cdots\right)\right\}$$

$$\bar{D}_{0-T} = \frac{D_0}{\lambda T}\left(\lambda T - \frac{\lambda^2 T^2}{2} + \frac{\lambda^3 T^3}{6} - \cdots\right)$$

$$\bar{D}_{0-T} = D_0 - \frac{D_0 \lambda T}{2} + \frac{D_0 \lambda^2 T^2}{6} - \cdots.$$

Where λT is small compared to 1, the higher order terms can be neglected. Then

$$\bar{D}_{0-T} \cong D_0 - \frac{D_0 \lambda T}{2}.$$

But $D_0 \lambda T$ approximates $D_0 - D_T$ for small values of λT. Therefore

$$\bar{D}_{0-T} \cong \frac{D_0 + D_T}{2}.$$

APPENDIX C

Sample protocol and calculations

No. 70 41 year old white woman 5′5½″, 79.6 kg Surface area = 1.86 m^2. Dose 49.7 μc i.v. at 2:02 p.m., 1/22/51.

Time after injection	Activity in μc over neck
Background	.76 μc
1 minute	7.70 μc
2 minutes	7.47 μc
3 minutes	7.61 μc
—	—
29 minutes	9.75 μc
30 minutes	9.90 μc
31 minutes	9.90 μc

Urine passed at 2:36 p.m. (29 minute specimen) contained 4.25 μc.

Calculations

Initial extrathyroidal level in neck (E) = 7.47 μc − .76 μc = 6.71 μc.

Observed increase in activity over neck (O) = 9.90 μc − 7.47 μc = 2.43 μc.

29 minute urinary excretion (U) = 4.25 μc.

Dose administered (D) = 49.7 μc.

Substituting these values in equation 2:

Thyroid uptake (Th)

$$= \frac{(2.43)(49.7) + (6.71)(4.25)}{49.7 - 6.71} = 3.48\ \mu c.$$

Dose retained at end of half hour ($D_{\frac{1}{2}\,hr}$)

$$= 49.7 - (3.48 + 4.25)\ \mu c,$$
$$= 41.97\ \mu c.$$

Average dose retained ($\bar{D}_{0-\frac{1}{2}\,hr}$):

(a) Determination of arithmetic mean dose:

From equation 7: $\bar{D}_{0-\frac{1}{2}\,hr} = \frac{49.7 + 41.97}{2} = 45.8\ \mu c$

(b) Determination of approximate integrated dose:
From equation *c* in Appendix A:

$$\lambda T = -\ln \frac{41.97}{49.7} = 0.167$$

From equation *b*:

$$\bar{D}_{0-\frac{1}{2}\,hr} = \frac{49.7(1 - e^{-0.167})}{0.167} = 45.8\ \mu c.$$

Substituting the arithmetic mean dose from (*a*) in the final clearance formula (equation *8* or *9*)

$$Cl_{thyroid} = \frac{3.48}{45.8} \times \frac{.20 \times 10^3}{30} \times 79.6 = 40.4\ ml/min.$$
$$= 37.6\ ml/min./1.73\ m^2$$

$$Cl_{renal} = \frac{4.25}{45.8} \times \frac{.20 \times 10^3}{29} \times 79.6 = 51\ ml/min.$$
$$= 47.5\ ml/min./1.73 m^2$$

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THE ABSORPTION AND EXCRETION OF TOCOPHEROL IN LAENNEC'S CIRRHOSIS [1]

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The plasma tocopherol level is often low in cirrhosis (1, 2). Whether such low values reflect alterations in the absorption, utilization or excretion of tocopherol related to liver damage, or are the result of a decreased dietary intake is not known. Previous attempts to investigate tocopherol metabolism in liver disease have been limited to tests of tocopherol tolerance based on plasma curves (1, 2) and have shed little light on the problem, largely because tolerance tests cannot be interpreted in terms of absorption and utilization without parallel studies of excretion and storage. The present report is concerned with the results of such studies in Laennec's cirrhosis. These have been made possible by the recent development of analytical methods for the determination of tocopherol in excreta.

In a preliminary investigation (1) of a group of subjects with a variety of liver diseases, including Laennec's cirrhosis, it was found that the plasma tocopherol level was significantly lower than in young healthy adults, but that it did not differ significantly from that of randomly-selected, hospitalized convalescent controls without evidence of liver disease. Moreover, there was no correlation between the plasma level and the degree of hepatic dysfunction, suggesting that low levels might be related to extrahepatic factors. Oral tocopherol tolerance tests were carried out to exclude impairment of absorption as one of these factors. These revealed relative flat plasma curves, but the lack of any correlation between the height of the curve and the original plasma level did not appear to be consistent with the interpretation that low plasma concentrations were the result of impaired absorption. Attempts to investigate tocopherol utilization by means of parenteral tolerance tests were unsuccessful, because none of the available preparations of tocopherol could be used intravenously, and none was freely absorbed from muscle. No definite conclusions could be drawn from these observations, but it was suggested that the low plasma levels seen in some subjects with liver disease might be the result of a reduced dietary intake of tocopherol.

The present study was limited to Laennec's cirrhosis, since there was reason to believe that alterations in tocopherol metabolism might differ in the various hepatic disease groups previously investigated. The results indicate that there is no impairment of tocopherol absorption or storage in the cirrhotic, and suggest that low plasma tocopherol levels are probably indicative of depleted tissue stores related to a deficient dietary intake.

MATERIAL AND METHODS

Subjects. The combined study of tocopherol saturation, tolerance and excretion described below was carried out in 11 subjects with Laennec's cirrhosis, eight normal healthy adults, and seven randomly-selected, hospitalized, convalescent controls. Additional data on plasma tocopherol concentration and on tocopherol tolerance tests were obtained in 22 other cirrhotics, 23 normals, and 53 convalescents.

The diagnosis of Laennec's cirrhosis was clearly evident from the clinical and laboratory findings in every instance, and was confirmed by needle biopsy of the liver in all but one case. On the whole the disease was in an advanced stage when these studies were carried out. The normal healthy adults were medical students, physicians, nurses, and laboratory technicians between the ages of 20 and 40. The hospitalized controls were convalescent from a variety of diseases at the time of the study. Liver disease was excluded in both control groups on the basis of the history and physical findings and the result of multiple liver function tests.

Diets. The cirrhotics were on an experimental diet which provided 1 gram of protein per kilogram, 100 grams of fat, and sufficient carbohydrate to raise the total intake to 30 calories per kilogram of body weight. The total tocopherol content of several such diets was calculated from the tables published by Harris, Quaife and Swanson

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(3) and was found to range between 17.87 and 25.97 mg., averaging 21.03 mg. per day.

The normal controls, with few exceptions, ate their meals in the staff dining room of the hospital. The tocopherol content of the diet provided there on five randomly-selected days varied between 22.14 and 42.73 mg., averaging 33.53 mg. per day.

The convalescent controls were all on the routine hospital diet while under investigation. Its tocopherol content was found to range between 25.52 and 43.50 mg. on five randomly-selected days, and averaged 30.76 mg.

Analytical techniques. Plasma tocopherol concentration was determined on oxalated venous blood by the method of Quaife and Biehler (4). Due to the large volume of plasma required it was not possible to carry out analyses in duplicate. Recoveries of 98.7 to 101.5 per cent were obtained when d-alpha-tocopherol was added to serum in amounts equivalent to 0.5 to 2.0 mg. per cent.

Fecal tocopherol was determined by a modification (5) of the method described by Quaife and Harris (6) for the analysis of total tocopherol in foods. In brief the specimens were homogenized, quick-frozen in dry-ice and alcohol, dehydrated under vacuum, and then extracted with hot ethanol in a Soxhlet apparatus. The extracted lipids were then transferred to petroleum ether, evaporated, and then distilled under high vacuum in an analytical molecular still. The distillate was taken up in ethanol, hydrogenated to remove non-tocopherol reducing substances, and then analyzed for total tocopherol photometrically, employing the Emmerie-Engel ferric chloride reduction-bipyridine reaction. All determinations were carried out in duplicate samples, which did not vary by more than ± 3 per cent. Recoveries of 98 to 103 per cent were obtained following molecular distillation of pure d-alpha-tocopherol in amounts ranging from 10 to 1,000 gamma. On addition of d-alpha-tocopherol to 100 gram aliquots of feces, recoveries varied from 92 to 99 per cent with additions of 25 to 1,000 mg., but fell to 73 to 89 per cent when the addition was as small as 10 mg.

The method employed in analyzing the plasma and feces for tocopheryl esters was based on a saponification technique carried out in an atmosphere of nitrogen, following preliminary evacuation of air from the samples and reagents (5).

All samples of plasma and feces were stored in a dry-ice refrigerator at temperatures well below − 20° C. and were usually analyzed within a few days of their collection. It has been shown that tocopherol is stable under these conditions for at least eight weeks (7).

Tocopherol tolerance tests. Following the administration of 500 mg. of dl-alpha-tocopheryl acetate (457 mg. of free tocopherol) in tablet form,[3] specimens of blood were drawn for tocopherol analysis at 0, 3, 6, 9, 12, and 24 hours. The test was begun in the early morning with the subject in the fasting state, but only the 0 and 24 hour specimens were collected under these conditions, the subject being allowed to eat regularly during the remainder of the test. In a previous study (1) it was shown that the diets employed had no significant effect on the plasma tocopherol level.

Combined saturation, tolerance, and excretion study. Each of the subjects was studied for six consecutive three-day periods. The plasma tocopherol concentration was determined daily, while fecal analyses were carried out on pooled three-day collections marked off with orally administered carmine-red.

Oral tocopherol tolerance tests were carried out on day three, at the beginning of Period II, and again on day ten during Period IV. The first test followed a three-day control period of the diet alone. The second test followed the administration of 4000 mg. of dl-alpha-tocopheryl acetate (3,656 mg. of free tocopherol) given as follows: day three 500 mg., days four through eight 200 mg. t.i.d., day nine none, and day ten 500 mg.

The following is a summary of the procedures carried out:

Day	Plasma tocopherol	Fecal tocopherol	Tocopheryl acetate administered per period	Tocopherol acetate administered daily
			mg.	*mg.*
0	—			0
1	*			0
2	*	Period I	0	0
3	*			500 Tolerance test I
4	*			600
5	*	Period II	1,700	600
6	*			600
7	*			600
8	*	Period III	1,800	600
9	*			0
10	*			500 Tolerance test II
11	*	Period IV	500	0
12	*			0
13	*			0
14	*	Period V	0	0
15	*			0
16	*			0
17	*	Period VI	0	0
18	*			

In addition to these studies, complete urine collections were made daily for creatine and creatinine analysis, a sample of blood was tested for dialuric acid hemolysis, and, in the cirrhotic group, a section of liver obtained by needle biopsy was stained for acid-fast ceroid in an attempt to demonstrate physiological evidence of vitamin E deficiency. The results of these studies will be reported elsewhere.

RESULTS

Fasting plasma tocopherol level. The mean plasma tocopherol concentration was 1.22 ± 0.35 mg. per cent in the normal healthy adults, 0.92 ± 0.32 mg. per cent in the cirrhotics, and 0.97 ± 0.33 mg. per cent in the convalescent controls. The concentration in the cirrhotic group was significantly lower than in the normal healthy adults, but

[3] "Ephynal Acetate," obtained through the courtesy of Dr. R. J. Floody, Hoffmann-La Roche, Inc., Nutley, N. J.

TABLE I

Fasting plasma tocopherol concentration

	Number of observations	Number of subjects	Maximum	Minimum	Mean	Standard deviation
			mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*
Total observations*						
normals	41	31	2.00	0.62	1.22	±0.35
cirrhotics	69	33	1.70	0.29	0.92	±0.32
convalescents	78	60	2.01	0.35	0.97	±0.33
Individual observations† (initial values only)						
normals	31	31	2.00	0.64	1.22	±0.33
cirrhotics	33	33	1.40	0.29	0.86	±0.29
convalescents	60	60	2.01	0.35	0.99	±0.35

* Mean value for normals significantly greater than that of cirrhotics ($t = 3.80$, $p<0.01$) and convalescents ($t = 4.73$, $p>0.01$). No significant difference between means for cirrhotics and convalescents ($t = 0.88$, $p>0.20$).

† Mean value for normals significantly greater than that of cirrhotics ($t = 3.26$, $p<0.01$) and convalescents ($t = 4.62$, $p>0.01$). No significant difference between means for cirrhotics and convalescents ($t = 1.89$, $p>0.05$).

TABLE II

Relation of fasting plasma tocopherol concentration to tocopherol administration

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Tocopherol administered (mg.)	0	0	457	548	548	548	548	548	0	457	0	0	0	0	0	0	0	0
	mg. per cent																	
Normals																		
1	0.64	0.69	0.62	0.82	—	0.98	1.15	1.04	1.15	1.04	0.64	—	0.85	0.82	0.73	0.66	0.64	0.62
2	0.90	0.90	0.89	1.56	—	1.70	1.80	1.92	1.96	1.94	1.91	—	1.76	1.68	1.40	1.18	0.92	0.89
3	0.90	0.94	0.92	1.06	1.37	—	1.69	1.63	2.12	1.67	1.48	1.24	—	0.95	1.18	0.92	0.94	0.90
4	1.06	1.12	1.09	1.58	1.68	1.74	1.82	1.78	1.84	1.64	1.64	1.35	1.37	1.28	1.20	1.14	1.10	1.08
5	1.20	1.22	1.25	1.64	2.13	2.26	—	2.36	2.34	1.86	1.68	1.48	1.32	—	1.20	1.22	1.21	1.20
6	1.37	1.34	1.36	1.64	1.68	1.74	1.80	1.76	1.94	1.90	1.84	1.64	1.53	1.52	1.42	1.38	1.34	1.30
7	1.73	1.72	1.78	2.82	3.12	3.25	—	3.02	3.13	2.86	2.08	1.86	1.89	—	1.84	1.74	1.77	1.70
8	1.75	1.72	1.74	2.12	2.21	2.18	—	2.46	2.38	2.26	2.44	1.86	1.74	—	1.78	1.70	1.76	1.74
Mean	1.19	1.21	1.21	1.66	2.03	1.98	1.65	2.00	2.11	1.90	1.71	1.60	1.49	1.25	1.34	1.24	1.21	1.18
Cirrhotics																		
9	0.34	—	0.38	0.44	0.51	0.62	—	—	1.28	1.33	1.17	1.04	0.74	0.80	—	0.74	0.64	0.58
10	0.39	0.52	0.42	0.74	—	—	1.03	1.36	1.42	1.34	1.10	0.90	—	0.69	—	0.55	0.54	0.56
11	0.54	0.56	0.58	0.85	—	1.09	1.35	1.36	1.16	1.01	1.09	—	0.93	1.04	0.92	0.78	0.62	0.55
12	0.60	0.52	0.61	0.78	—	1.78	2.48	1.87	—	1.46	1.28	—	0.89	0.90	1.02	0.96	0.94	0.92
13	0.92	0.90	0.94	1.91	2.16	1.95	2.02	2.08	1.99	1.95	1.56	1.61	1.58	—	1.64	1.56	1.50	1.48
14	0.94	0.89	0.86	1.30	1.51	1.74	1.86	2.57	—	1.90	1.47	2.15	1.67	1.38	1.24	—	1.36	1.38
15	1.05	0.98	1.06	—	2.16	2.06	2.26	2.34	—	2.04	1.64	1.60	1.54	1.48	1.28	—	1.16	1.13
16	1.09	1.10	1.01	1.94	2.05	—	2.70	—	2.80	2.58	2.18	2.11	—	—	1.94	1.71	1.56	1.52
17	1.10	1.14	1.08	1.98	—	1.78	1.86	1.95	1.80	1.44	1.84	—	1.40	—	—	1.20	—	1.20
18	1.12	1.16	1.09	1.41	1.56	1.80	1.89	1.78	—	1.37	1.58	1.38	1.30	1.27	0.95	—	1.04	1.14
19	1.36	1.32	1.38	2.12	2.00	1.75	1.78	2.10	—	2.18	1.86	1.64	1.53	1.46	1.34	—	1.36	1.34
Mean	0.86	0.91	0.85	1.35	1.71	1.62	1.92	1.93	1.74	1.69	1.53	1.55	1.29	1.13	1.29	1.07	1.07	1.07
Convalescents																		
20	0.63	0.62	0.66	0.74	0.82	0.90	0.98	1.14	1.20	0.96	0.94	0.89	0.90	—	0.92	0.90	0.94	0.92
21	0.71	0.75	0.72	1.26	1.38	1.44	—	1.46	1.58	1.40	1.32	1.14	0.86	0.82	0.72	—	0.74	0.70
22	0.72	0.72	0.74	0.76	0.80	0.92	1.22	1.30	—	1.20	1.18	1.10	0.94	0.96	0.84	—	0.77	0.74
23	0.76	0.74	0.78	0.94	1.02	1.14	1.22	—	—	1.20	1.24	1.12	0.94	0.86	—	—	0.63	0.78
24	0.86	0.87	0.85	0.91	—	1.80	1.88	—	2.13	1.80	1.80	—	—	—	1.14	—	0.85	—
25	0.98	0.94	1.05	1.86	1.72	1.95	—	2.18	2.02	2.11	2.66	2.40	—	1.71	—	1.64	1.58	1.28
26	1.09	1.02	1.12	1.84	1.98	2.03	1.90	1.96	1.86	1.78	1.69	1.59	1.46	—	—	1.16	—	1.10
Mean	0.82	0.81	0.85	1.19	1.29	1.45	1.44	1.61	1.76	1.49	1.55	1.37	1.02	1.09	0.91	1.23	0.92	0.92

did not differ significantly from that in the convalescent controls (Table I). However, it will be noted that there was considerable overlapping of values in all three groups, and that low values were by no means a constant finding in cirrhosis. Moreover, in the cirrhotic group there was no correlation between the plasma level and the state of hepatic function, as judged by the serum concentrations of albumin ($n = 27$, $r = +0.330$, $p > 0.05$) and bilirubin ($n = 41$, $r = -0.043$, $p > 0.05$).

These observations suggested that extrahepatic factors might be more important in determining the plasma tocopherol level in cirrhosis than the state of hepatic function.

Effect of tissue saturation on the plasma tocopherol level. An attempt was made to saturate the tissues with tocopherol to determine whether low plasma levels were indicative of relatively depleted tissue stores. The amount of tocopherol administered, 3.656 grams in eight days, exceeded the estimated total body content (8), so that appreciable tissue saturation probably occurred, even though simultaneous fecal studies revealed that only 60 to 70 per cent was absorbed.

During the administration of tocopherol the plasma level rose steadily, usually reaching a peak on the fifth and sixth day (Table II). At the peak neither the absolute values attained nor the increases above the initial levels differed significantly in the three groups of subjects (Table III). However, following the withdrawal of tocopherol, there was a prompt fall to the pretreatment level in the normal group, while the level remained elevated in eight of the 11 cirrhotics and two of the seven convalescent controls (Table II). The mean increase in concentration eight days following cessation of tocopherol administration was statistically significant in the cirrhotic group, but not in the convalescent controls (Table III).

The readiness with which the plasma concentration could be raised by means of orally administered tocopherol and the sustained character of the increase following presumed saturation of the tissues suggested that the low plasma levels in cirrhosis were probably more closely related to tissue depletion than to any impairment of absorption or utilization. A similar sustained increase in plasma tocopherol was observed following the administration of tocopherol to individuals

TABLE III

Effect of daily tocopherol administration on fasting plasma tocopherol concentration

	Number of subjects	Maximum	Minimum	Mean	Standard deviation
Maximum increase in plasma tocopherol over control level, *mg. per cent*					
normals	8	1.20	0.50	0.93*	±0.33
cirrhotics	11	1.90	0.67	1.17*	±0.40
convalescents	7	1.67	0.48	0.91*	±0.41
Maximum concentration of plasma tocopherol attained, *mg. per cent*					
normals	8	3.25	1.15	2.14*	±0.56
cirrhotics	11	2.80	1.33	2.05*	±0.48
convalescents	7	2.66	1.20	1.73*	±0.52
Day of tocopherol administration on which maximum concentration was attained					
normals	8	6	3	5.1*	±1.2
cirrhotics	11	7	4	5.4*	±0.8
convalescents	7	8	3	6.0*	±1.6
Difference between tocopherol concentration 8 days following cessation of tocopherol administration and initial control level					
normals	8	0.00	−0.06	−0.02†	±0.02
cirrhotics	11	+0.56	−0.01	+0.21†	±0.20
convalescents	7	+0.29	−0.03	+0.08	±0.13

* Difference between means not statistically significant.

† Difference between normals and cirrhotics highly significant ($t = 3.71$, $p < 0.01$); difference between normals and convalescents, or cirrhotics and convalescents not statistically significant.

TABLE IV

Results of oral tocopherol tolerance tests (500 mg. dl-alpha-tocopheryl acetate)

	Number of subjects	Maximum	Minimum	Mean	Standard deviation
Maximum increase in plasma tocopherol concentration, *mg. per cent*					
normals	8	2.00	0.20	0.83	±0.57
cirrhotics	26	1.86	0.00	0.70*	±0.12
convalescents	28	3.25	0.12	1.12*	±0.85
Mean increase in plasma tocopherol concentration, *mg. per cent* (calculated from area of curves)					
normals	8	0.89	0.13	0.48†	±0.27
cirrhotics	26	1.17	0.00	0.39†	±0.29
convalescents	28	1.96	0.00	0.57†	±0.51
Maximum concentration of plasma tocopherol during tolerance test, *mg. per cent*					
normals	8	3.09	0.82	2.04†	±0.76
cirrhotics	26	2.64	0.56	1.65†	±0.60
convalescents	28	4.98	0.76	2.03†	±0.99

* Difference between cirrhotics and convalescents statistically significant ($t = 2.59$, $p < 0.02$); difference between cirrhotics and normals, or convalescents and normals not statistically significant.

† Difference between means not statistically significant.

whose previous intake had been low and whose tissues had presumably been depleted (9). Judging from the results of animal feeding experiments (10) it may be presumed that the tissue stores were likewise increased by tocopherol administration in the normal and convalescent controls, even though there was no sustained increase in plasma concentration. These findings suggest that there may be mechanisms tending to stabilize the plasma tocopherol level within the normal range.

Fractionation of plasma tocopherol. Since tocopheryl esters fail to react in the Emmerie-Engel reaction, on which our analytical procedures were based, they would go undetected leading to falsely low values. It was necessary, therefore, to exclude their presence by analyzing the plasma following saponification. By this method, no tocopheryl esters could be demonstrated in the plasma, even after large doses of tocopheryl acetate were administered. Thus, in a cirrhotic with

TABLE V

Comparison of oral tocopherol tolerance tests before and after the administration of 3.5 grams of dl-alpha-tocopheryl acetate over a six-day period

	Number of subjects	Maximum increase in plasma tocopherol concentration				Mean rise in plasma tocopherol concentration				Maximum plasma tocopherol at height of curve			
		Maximum	Minimum	Mean	Standard deviation	Maximum	Minimum	Mean	Standard deviation	Maximum	Minimum	Mean	Standard deviation
		mg. per cent				*mg. per cent*				*mg. per cent*			
Normals	8												
before		2.00	0.20	0.83*	±0.57	0.89	0.13	0.48†	±0.27	3.09	0.82	2.04*	±0.76
after		1.32	0.06	0.58*	±0.38	0.36	0.01	0.20†	±0.11	3.75	1.27	2.48*	±0.70
Cirrhotics	11												
before		1.42	0.38	0.76*	±0.37	0.87	0.16	0.44†	±0.24	2.78	0.76	1.62†	±0.62
after		1.70	0.00	0.61*	±0.34	0.70	0.00	0.18†	±0.18	3.14	1.46	2.28†	±0.65
Convalescents	7												
before		1.12	0.12	0.57*	±0.39	0.64	0.01	0.33*	±0.24	2.14	0.76	1.40†	±0.49
after		1.06	0.22	0.67*	±0.08	0.41	0.06	0.19*	±0.10	2.84	1.42	2.16†	±0.58

* Difference between means not statistically significant.

† Difference between means statistically significant: mean rise in normals, $t = 2.56$, $p < 0.05$; in cirrhotics, $t = 2.76$, $p < 0.02$; maximum plasma tocopherol in cirrhotics, $t = 2.35$, $p < 0.05$; in convalescents, $t = 2.45$, $p < 0.05$.

low plasma levels of 0.37, 0.42, and 0.39 mg. per cent on successive days while receiving 600 mg. of dl-alpha tocopheryl acetate daily, there were recoveries of only 0.20, 0.28, and 0.22 mg. per cent following saponification. Similarly in a normal subject with a high plasma level of 2.78 mg. per cent during a similar period of tocopheryl acetate administration, only 1.65 mg. per cent could be recovered following saponification. These recoveries, representing 54 to 67 per cent of the original tocopherol present, were even smaller than those obtained following saponification of comparable concentrations of pure d-alpha-tocopherol in alcohol.

Other investigators (11, 12) have likewise failed to demonstrate the presence of tocopheryl esters in the plasma of normal animals following the oral administration of tocopheryl acetate or phosphate.

Oral tocopherol tolerance tests. To further investigate the possibility that the low plasma tocopherol levels in cirrhosis were more closely related to depleted tissue stores than to impaired absorption or hepatic dysfunction, the response of the plasma level to a single test dose of tocopheryl acetate was studied both before and after tissue saturation.

Before saturation, the cirrhotics showed a smaller rise in plasma tocopherol concentration than either the normal or convalescent controls (Table IV). However, the difference between the cirrhotic and normal subjects was not statistically significant, and when the tolerance curves were compared on the basis of the mean rise in concentration, calculated from the areas under the

TABLE VI

Relation of fecal tocopherol excretion to tocopherol intake

	Number of subjects	Tocopherol intake			Fecal tocopherol excretion			
		Dietary	Fed as acetate	Total	Maximum	Minimum	Mean	Standard deviation
		mg. per 3-day period			*mg. per 3-day period*			
Period I								
normals	6	100.6	0	100.6	74.37	48.39	64.85	± 8.79
cirrhotics	6	63.1	0	63.1	35.21	12.26	22.23*	± 8.96
convalescents	5	92.3	0	92.1	62.58	36.65	49.29†	± 8.44
Period II								
normals	6	100.6	1,553.8	1,654.4	1,138.80	797.04	929.36	±108.4
cirrhotics	6	63.1	1,553.8	1,616.9	644.49	292.69	507.65*	±119.6
convalescents	5	92.3	1,553.8	1,646.1	982.68	742.73	861.54	± 79.0
Period III								
normals	6	100.6	1,645.2	1,745.8	1,248.56	969.15	1,070.79	± 98.8
cirrhotics	6	63.1	1,645.2	1,708.3	840.09	337.34	634.85*	±157.6
convalescents	5	92.3	1,645.2	1,737.5	1,240.83	950.10	1,080.24	±105.9
Period IV								
normals	6	100.6	457.0	557.6	429.57	275.10	330.22	± 49.5
cirrhotics	6	63.1	457.0	520.1	193.44	126.99	158.83*	± 24.7
convalescents	5	92.3	457.0	549.3	286.08	212.59	252.71	± 24.6
Period V‡								
normals	6	100.6	0	100.6	100.31	57.99	79.97	± 14.70
cirrhotics	6	63.1	0	63.1	62.27	36.90	47.38*	± 8.42
convalescents	5	92.3	0	92.3	84.33	53.75	68.12	± 10.94
Period VI§								
normals	6	100.6	0	100.6	70.48	54.78	63.25	± 5.32
cirrhotics	6	63.1	0	63.1	48.39	34.53	38.53*	± 5.03
convalescents	5	92.3	0	92.3	75.25	45.71	60.08	± 9.70

* Excretion in cirrhotics significantly lower than in normals and convalescents (Period I, $t = 7.61$ and 4.66, respectively; Period II, $t = 5.86$ and 5.32; Period III, $t = 5.25$ and 5.06; Period IV, $t = 8.06$ and 5.68; Period V, $t = 4.18$ and 3.00; Period VI, $t = 7.14$ and 3.94; "t" value of 3.17 = $p<0.01$; t, 3.00 = $p<0.02$).

† Excretion in convalescents significantly lower than in normals during Period I only ($t = 2.71$, $p<0.05$).

‡ Excretion during Period V significantly greater than in Period I in convalescents and in cirrhotics ($t = 2.72$, $p<0.05$ and $t = 4.33$, $p<0.01$, respectively). Difference between Periods I and V in normals of borderline significance ($t = 1.98$, $p<0.10$, >0.05).

§ Excretion during Period VI significantly greater than in Period I in cirrhotic group only ($t = 2.38$, $p<0.05$).

TABLE VII

Percentage of ingested tocopherol recovered in the feces

	Number of subjects	Mg. tocopherol ingested per period*	Per cent tocopherol recovered in feces			
			Maximum	Minimum	Mean	Standard deviation
Period I						
normals	6	100.6	73.9	48.1	64.4	± 9.2
cirrhotics	6	63.1	55.8	19.4	35.2†	±14.2
convalescents	5	92.1	67.9	39.8	53.5	± 9.0
Period II						
normals	6	1,654.4	68.8	48.2	56.2	± 6.4
cirrhotics	6	1,616.9	39.9	18.1	31.4†	± 7.5
convalescents	5	1,646.1	59.7	45.1	52.3	± 5.0
Period III						
normals	6	1,745.8	71.5	55.5	61.3	± 6.0
cirrhotics	6	1,708.3	49.2	19.7	37.2†	± 9.0
convalescents	5	1,737.5	71.4	54.7	62.2	± 5.9
Period IV						
normals	6	557.6	77.0	49.3	59.2	± 9.0
cirrhotics	6	520.1	37.2	24.4	30.6†	± 4.4
convalescents	5	549.3	52.1	38.7	46.0‡	± 4.5
Period V§						
normals	6	100.6	99.7	57.6	79.5	±14.6
cirrhotics	6	63.1	98.7	58.5	75.1	±13.2
convalescents	5	92.3	91.6	58.4	74.0	±11.7
Period VI§						
normals	6	100.6	70.1	54.5	62.9	± 4.9
cirrhotics	6	63.1	76.7	54.7	61.1	± 7.7
convalescents	5	92.3	81.7	49.6	65.2	±10.6

* Calculated content of diet plus tocopherol administered as acetate (see Table VI).

† Excretion in cirrhotics significantly less than in normals or convalescents during Periods I through IV (Period I, $t = 3.89$ and 2.36, respectively; Period II, $t = 5.63$ and 5.06; Period III, $t = 4.98$ and 5.06; Period IV, $t = 6.39$ and 5.18; "t" value of 3.17 = $p < 0.01$).

‡ Excretion in convalescents significantly less than in normals during Period IV only ($t = 2.88$, $p < 0.02$).

§ Excretion during Period V significantly greater than in Period I in cirrhotics ($t = 4.36$, $p < 0.01$) and convalescents ($t = 2.75$, $p < 0.05$); difference of borderline significance in normals ($t = 1.96$, $p < 0.10 > 0.05$); excretion during Period VI significantly greater than in Period I in cirrhotics only ($t = 3.51$, $p < 0.01$).

curves measured with a planimeter, or on the basis of the maximum absolute values attained at the peak, no significant differences were discerned between any of the groups. Moreover, in the cirrhotic group no significant correlations could be demonstrated between the magnitude of the rise in plasma tocopherol concentration and the initial level ($n = 25$, $r = +0.274$, $p > 0.05$), or the state of hepatic function, as judged by the concentrations of serum albumin ($n = 19$, $r = -0.160$, $p > 0.05$) and bilirubin ($n = 25$, $r = -0.080$, $p > 0.05$).

Following saturation of the tissues with tocopherol, the tolerance curves in the three groups no longer differed, whether compared on the basis of the maximum or mean rise in plasma tocopherol, or on the basis of the absolute values attained at the peak of the curves (Table V). Unfortunately, in the three small groups of subjects, randomly-selected for testing before and after saturation, there were no differences between the tolerance curves before saturation (Table V), so that the uniformity of the curves observed after saturation could not be ascribed to a change in the state of the tissue stores.

When the tolerance tests carried out before and after saturation were compared (Table V), it was found that the rise in plasma tocopherol concentration was smaller after saturation than before, both in the normal and cirrhotic groups. The differences were not statistically significant when the comparison was based on the maximum increase in plasma tocopherol, but were significant when based on the mean rise calculated from the

TABLE VIII

Correlations between fecal excretion of tocopherol and the plasma tocopherol level, the results of the tocopherol tolerance test and the state of hepatic function

Subject	Fecal tocopherol, Period I*	Mean fasting plasma tocopherol*	Tocopherol tolerance test		Serum bilirubin	Serum albumin
			Maximum rise	Mean rise		
	mg. per 3-day period	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*gm. per cent*
Cirrhotic	12.26	0.36	0.38	0.21	6.82	1.71
Cirrhotic	12.51	1.11	0.90	0.60	1.10	2.70
Cirrhotic	18.37	0.90	0.44	0.16	1.58	2.20
Cirrhotic	22.62	0.58	0.45	0.28	2.72	2.39
Cirrhotic	32.42	1.03	1.42	0.38	12.46	1.79
Cirrhotic	35.21	0.44	0.54	0.35	3.58	1.48
Convalescent	36.65	0.73	0.02	0.01	0.74	
Convalescent	46.64	0.76	0.42	0.22	0.60	
Convalescent	48.03	0.64	0.60	0.22	0.82	
Normal	48.39	1.09	2.00	0.81	0.48	
Convalescent	52.54	0.86	0.12	0.09	—	
Normal	59.93	1.22	0.43	0.28	0.64	
Convalescent	62.58	0.73	0.70	0.51	0.84	
Normal	64.51	0.65	0.20	0.15	0.46	
Normal	69.81	1.74	1.11	0.89	0.60	
Normal	72.09	1.74	0.90	0.50	—	
Normal	74.37	0.92	0.23	0.13	0.74	

* Coefficient of correlation $r = +0.488$, $p < 0.05$.

areas under the curves. However, as might be expected from the higher plasma concentrations at the start of the post-saturation test, the maximum absolute values attained at the peak of the curves were significantly higher than before saturation.

It is difficult to account for the smaller rise in plasma tocopherol in the post-saturation tolerance test, unless it is assumed that there was an upper limit of concentration which could not be exceeded. If there was such a ceiling it varied in different individuals, since the maximum values observed ranged from 1.27 to 3.75 mg. per cent (Table V), and could have been determined by several factors, including the relative rates of tocopherol absorption in the intestine and deposition in the tissues, the rate of excretion in the bile or urine, and possibly, the capacity of the plasma to transport tocopherol.

The results of the tolerance tests must be regarded as inconclusive with respect to the problem of whether the low plasma tocopherol levels found in cirrhosis are related to impaired absorption, or to unsaturated tissue stores dependent on other factors.

Fecal excretion of tocopherol. To investigate the factor of absorption more directly, studies of fecal tocopherol excretion were undertaken. These revealed that the cirrhotics excreted less tocopherol than either the normal or convalescent controls, both during the control period and following the administration of tocopheryl acetate (Table VI). The convalescents also excreted less tocopherol than the normals, but the difference was not statistically significant, except during the control period.

The amount of tocopherol excreted was roughly proportional to the intake (Table VI), but it is evident from the data in Table VII that the proportion excreted in the cirrhotic group was significantly lower than in the normal or convalescent controls, both during the control period and during tocopherol administration. The convalescents also excreted a smaller fraction of ingested tocopherol than the normal controls, but the difference was not statistically significant, except in Period IV (Table VII).

Immediately following the cessation of tocopherol administration there was a sharp fall in fecal tocopherol excretion, but it was still significantly greater in Period V than in Period I in all groups (Table VI), so that the ratios of excreted to ingested tocopherol rose to unusually high levels (Table VII). In the succeeding period, however, the normal and convalescent subjects returned to their control levels, but the cirrhotics did not, so that in Period VI the proportion of ingested tocopherol excreted no longer differed in the three groups (Table VII).

There appeared to be a significant relationship between the tocopherol content of the feces during the control period and its concentration in the plasma, but no correlations could be demonstrated between fecal tocopherol and the state of hepatic function or the results of the tocopherol tolerance tests (Table VIII).

These findings strongly suggested that there was no impairment of absorption in the cirrhotics, and that, actually, when large doses of tocopherol were ingested the proportion absorbed was even greater than in the normal or convalescent controls. However, the validity of estimating tocopherol absorption from the results of fecal analyses still had to be established. In particular it was necessary to exclude the presence of esterified tocopherol in the feces, and to rule out the occurrence of significant destruction or synthesis of tocopherol in the intestinal tract.

Fractionation of fecal tocopherol. No tocopheryl esters could be demonstrated in the feces, either during the control period, or during periods of high tocopheryl acetate intake. Thus, 71 to 80 per cent of the original tocopherol found on distillation was recovered following saponification of the feces, values which compared favorably with recoveries following saponification of pure dl-alpha-tocopherol added to feces (Table IX).

TABLE IX

Recovery of tocopherol in feces following saponification

Subject	Period	dl-alpha tocopheryl acetate ingested	Tocopherol recovered on distillation	Tocopherol recovered following saponification	
		mg.	*mg.*	*mg.*	*per cent of amount found on distillation*
Cirrhotic	I	0	12.42	9.76	78.5
Normal	I	0	69.81	50.57	72.4
	II	1,700	1,138.80	916.62	80.5
	III	1,800	1,115.92	807.53	72.4
	IV	500	429.51	321.44	74.8
	V	0	72.99	53.40	73.2
	VI	0	65.79	47.03	71.5
Normal	I	0	48.62	36.02	74.1
	II	1,700	954.76	726.64	76.1
	III	1,800	1,150.12	877.31	76.3
	IV	500	354.45	259.90	73.3
	V	0	58.19	41.55	71.4
	IV	0	55.30	40.43	73.1

Recovery of dl-alpha-tocopheryl acetate added to 100 gm. aliquots of feces

Amount added	Recovered following saponification	
mg.	*mg.*	*per cent*
10.00	7.21	72.1
25.00	18.47	74.0
50.00	37.00	74.0
100.00	76.34	76.3
500.00	386.40	77.2
1,000.00	787.40	78.7

Destruction of tocopherol in feces and intestinal contents. M. K., a female cirrhotic with a plasma tocopherol level of 0.80 mg. per cent, excreted 443.23 mg. of tocopherol in a three-day period during which she ingested 1,800 mg. of dl-alpha-tocopheryl acetate. Following incubation of the feces in a fine watery suspension at 37° C. for 24 hours, there was no loss of tocopherol, 454.62 mg. being recovered.

The contents of the lower jejunum were aspirated in N. R., a middle-aged negress with complete intestinal obstruction, who had no food by mouth and who had been maintained on tocopherol-free parenteral fluids for a period of two weeks. The fluid had a pH of 6.5 and contained 0.24 mg. per cent of tocopherol. Following incubation at 37° C. for 24 hours the concentration fell insignificantly to 0.22 mg. per cent. When dl-alpha-tocopheryl acetate was added at a concentration of 2.0 gm. per cent, the ester was promptly broken down to free tocopherol, but there was no significant loss of tocopherol even after 48 hours of incubation (Table X).

TABLE X

Stability of tocopheryl acetate and esterase activity in incubated jejunal contents aspirated from a subject with intestinal obstruction; dl-alpha-tocopheryl acetate added in a concentration of 2,000 mg. per 100 ml. (equivalent to 1,828 mg. per cent of free tocopherol)*

Incubation at 37° C.	Stability of tocopheryl acetate		Esterase activity	
hours	*mg. free tocopherol recovered following saponification*	*per cent of original acetate recovered*	*mg. free tocopherol recovered before saponification*	*per cent of original acetate hydrolyzed*
1	1,689	92.4	50	2.7
2	1,683	92.1	165	9.0
4	1,689	92.4	368	20.1
8	1,677	91.7	646	35.3
24	1,665	91.1	1,161	63.5
48	1,653	90.4	1,347	73.7

*Original content of free tocopherol 0.24 mg. per cent.

The stability of tocopherol and its esters in incubated feces and jejunal fluid suggested that there was no significant destruction of tocopherol in the intestinal tract. Tocopherol synthesis was not excluded, but it could not have been a signifi-

cant factor in any of the experimental periods when there was a five- to 20-fold increase in fecal tocopherol following tocopherol administration. Moreover, there is no convincing evidence of tocopherol synthesis in experimental animals. Pindborg (13) reported that synthesis occurred in rats, but his animals were on a stock diet and the more likely possibility, that the tocopherol found in the feces represented an unabsorbed fraction of ingested tocopherol, was not considered.

Urinary excretion of tocopherol. The amount of tocopherol found in the urine was insignificant, even following the administration of large doses of tocopherol. Thus, L. B., a convalescent control, excreted a total of 0.40 mg. per day while on a normal diet, and 0.26 mg. following the ingestion of 1200 mg. of dl-alpha-tocopheryl acetate. Similarly, a cirrhotic, A. S., excreted 0.93 mg. per day during the control period and 0.56 mg. following the administration of a similar dose of tocopheryl acetate.

It was concluded that these negligible losses in the urine had no influence on the concentration of tocopherol in the plasma or feces, or on the results of the saturation and tolerance tests.

COMMENT

It is highly probable that low concentrations of tocopherol in the plasma are indicative of a similar decrease in the tissues. The sustained rise in the plasma level of cirrhotics following saturating doses of tocopherol (Table III), and the low concentrations of tocopherol found in cirrhotic liver tissue (2) are confirmatory evidence of such a relationship. The failure of saturating doses of tocopherol to produce a similar rise in plasma concentration in convalescents appears to be inconsistent, but the marked difference between the amounts of tocopherol absorbed by convalescents and cirrhotics during the saturation test (Tables VI and VII) probably accounts for this discrepancy.

The possible causes for tocopherol depletion in cirrhosis to be considered include impairment of absorption, increased utilization or destruction in the tissues and a decreased dietary intake of tocopherol.

The data on fecal excretion provide convincing evidence that there is no impairment of tocopherol absorption in cirrhosis, and the observation that continuous tocopherol administration produces as great an increase in plasma concentration in cirrhotics as in normal subjects (Table III) is confirmatory.

The validity of estimating tocopherol absorption from balance studies, based on measurements of intake and fecal excretion, rests on two assumptions, that there is no significant loss of tocopherol by excretion in the urine or by destruction in the intestinal tract, and that there is no significant enteric synthesis of tocopherol. The evidence presented satisfactorily excludes significant losses of tocopherol in the urine, but the results of the *in vitro* incubation experiments to rule out tocopherol destruction in the intestinal tract, while suggestive, are by no means conclusive and still need confirmation. The possibility that tocopherol is synthesized in the intestinal tract has not been ruled out, but appears unlikely. The only contradictory evidence (13) is not convincing and has not been confirmed. However, even if tocopherol synthesis should prove to be a significant factor, it is unlikely that it introduced any serious errors in the present balance experiments, since fecal tocopherol excretion was increased five- to 20-fold over the basal level during periods of tocopherol administration (Table VI).

The curious observation that cirrhotics apparently absorb a larger fraction of administered tocopherol than normal subjects (Table VII) warrants careful consideration. Conceivably the amount of tocopherol absorbed might be determined by its concentration in the plasma and tissues, as in the case of iron. However, this appears unlikely in view of the fact that a similar increase in tocopherol absorption is not seen in convalescent controls with equally low plasma concentrations (Table VII). A more likely possibility to be considered is that a significant fraction of the tocopherol absorbed is re-excreted via the biliary tract, and that under conditions of impaired hepatic function there is retention of this fraction to a greater or lesser extent. Most of the observed facts are compatible with this interpretation. These include the smaller fecal excretion of tocopherol in cirrhotics than in normal or convalescent controls, the greater effect of saturating doses of tocopherol on the plasma level in cirrhotics than in convalescents with similarly depleted tissue stores, the unusually large amount

of tocopherol excreted immediately following saturation in all groups (Period V, Tables VI and VII) and the persistence of this increased excretion for a longer period in cirrhotics than in normals or convalescents (Period VI, Tables VI and VII). Only the tendency toward flat plasma curves following a single test dose of tocopherol in cirrhosis appears to be inconsistent with the hypothesis of normal intestinal absorption and impaired biliary excretion of tocopherol. However, it is possible that this trend merely indicates a slower *rate* of absorption in cirrhosis, as suggested by the fact that the areas under the tolerance curves are normal, or a more rapid diversion of tocopherol from the plasma to relatively unsaturated tissues, as suggested by the more normal curves following saturation.

A preliminary investigation of the biliary excretion of tocopherol was carried out in four subjects with T-tube drainage of the common bile duct, to obtain more direct evidence of the re-excretion of absorbed tocopherol. It was found that the concentration of tocopherol in bile was of the same order of magnitude as in plasma, that the two concentrations varied independently, and that there was no increase in the bile concentration following the administration of large doses of tocopherol. These findings are consistent with the concept that a fraction of the tocopherol absorbed in the intestinal tract is re-excreted in the bile. However, it is not certain that the failure of the biliary concentration to rise following tocopherol administration was due to abnormal retention related to liver damage, since it was not possible to repeat this experiment in a normal subject.

The possibility that the low plasma tocopherol levels in cirrhosis are related to an increased rate of utilization or destruction of tocopherol appears unlikely in view of the sustained rise in the plasma level which follows saturating doses of tocopherol, and the lack of any correlation between the plasma level and the degree of hepatic dysfunction. On the other hand, these observations, and others, are compatible with the hypothesis that the low plasma tocopherol levels in cirrhosis are indicative of depleted tissue stores related to a decreased dietary intake of tocopherol. Certainly signs of other nutritional deficiencies are frequently observed in cirrhosis, and it is reasonable to suppose that tocopherol deficiency may also occur as a result of the reduced intake of food which so often precedes and accompanies the development of this disease. Qualitative and quantitative differences in the diet could well account for the inconstancy of low plasma tocopherol levels in cirrhosis. Similarly the low levels found in some convalescents could be related to a reduced intake of food during a prolonged antecedent illness.

SUMMARY

1. The mean plasma tocopherol level was significantly lower in subjects with Laennec's cirrhosis than in normal healthy controls, but did not differ significantly from that of hospitalized, convalescent controls with no evidence of liver damage. Moreover, low values were not a constand finding in cirrhosis, and could not be correlated with the degree of hepatic dysfunction present, suggesting that they were probably related to extrahepatic factors.

2. Large doses of tocopherol, equivalent to the estimated normal total body content, administered over an eight-day period, raised the plasma tocopherol to equally high levels in cirrhotics, normals and convalescent controls. However, following cessation of tocopherol administration, only the cirrhotics exhibited a sustained increase in plasma tocopherol over the control level. These findings suggested that there was no impairment of tocopherol absorption in cirrhosis and that low plasma levels were indicative of relatively unsaturated tissue stores.

3. Suggestive evidence was presented to establish the validity of estimating tocopherol absorption from measurements of tocopherol intake and fecal excretion. On the basis of such balance studies it was found that cirrhotics absorbed a higher proportion of administered tocopherol than either normal or convalescent controls, confirming the previous suggestion that low plasma levels were not the result of impaired tocopherol absorption. Several possible explanations for the apparently greater absorption of tocopherol in cirrhotics than in the controls were considered, but the data presented did not warrant any definite conclusions.

4. Tocopherol tolerance tests, based on the plasma response to a single test dose of tocopherol,

revealed that the cirrhotics tended to show curves that were lower than those of the controls, judging from the maximum increase in concentration over the basal level. However, when the curves were compared on the basis of the mean rise in concentration, estimated from the areas under the curves, the three groups did not differ significantly. Following saturation of the tissues with large doses of tocopherol no differences could be discerned between the three groups. Parallel fecal excretion studies indicated that flat tolerance curves could not be interpreted as evidence of impaired absorption. Other interpretations were considered, but it was not possible to reach any definite conclusions.

5. On the basis of the evidence presented it is suggested that low plasma tocopherol levels in cirrhosis are indicative of depleted tissue stores related to a reduced dietary intake of tocopherol.

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THE RELATIONSHIP OF PLASMA pH AND ANION PATTERN TO MERCURIAL DIURESIS[1]

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In 1925 Keith, Barrier and Whelan (1) noted that the diuretic potency of novasurol was increased when ammonium chloride was administered prior to the mercurial. This observation was subsequently extended to include potentiation of other mercurial diuretics by a variety of acidifying agents (2–5). Furthermore, potentiation has been shown to be synergistic for fluid loss induced by the combination exceeds the sum of the losses induced by the agents singly (2, 3, 5, 6). Potentiation has been variously ascribed to the following changes induced by the acidifying agents: increased acidity of the body fluids (2, 4, 5), increased acidity of the urine (7) and altered anion pattern of the blood plasma (4, 8, 9). Experiments described below suggest that the latter is the most significant factor and, possibly alone, can account for the observed increase in diuretic efficacy.

METHODS

All experiments were performed on two well trained female dogs, loosely restrained on a comfortable animal board. Creatinine for the measurement of glomerular filtration rate and para-aminohippurate for the measurement of minimum effective renal plasma flow were infused intravenously at a rate calculated to maintain plasma concentrations constant at optimum levels. The animals were fasted for 14 to 16 hours preceding an experiment, and 30 minutes prior to the start of the first collection period, 500 cc. of water were administered by stomach tube. After one or more control periods, salyrgan[2] was given intravenously. Since mercury diuresis was superimposed on water diuresis, little emphasis has been placed on urine flow. Rather diuretic efficacy has been assessed in terms of rates of excretion of sodium and chloride.

Urine was collected under paraffin oil by an indwelling urethral catheter and the bladder was emptied by firm, gentle, suprapubic pressure. Aliquots for carbon dioxide and pH determinations were withdrawn without exposure to air. Blood samples were collected by an indwelling needle in the femoral artery. That part of the sample to be used for pH and carbon dioxide determinations was drawn in an oiled syringe and handled anaerobically. Measurements of blood and urine pH were made at room temperature with a Cambridge condenser type glass electrode and corrected to 38° by subtracting .01 pH unit per degree difference in temperature. Bicarbonate was calculated from the carbon dioxide content and pH using the Henderson-Hasselbalch equation. Carbon dioxide was determined by the method of Van Slyke and Sendroy (10), chloride by the Van Slyke and Hiller modification of Sendroy's method (11), sodium and potassium on an internal standard flame photometer and creatinine (12) and para-aminohippurate (13) colorimetrically.

RESULTS

Three experiments on one dog are summarized in Table I. In each experiment 80 mg. of mercury as salyrgan were administered intravenously at zero time. The first experiment describes the more or less typical response of this animal to the mercurial diuretic alone and serves as a standard of reference with which the response under other conditions may be compared. In this experiment sodium excretion increased from 247 microequivalents per minute in the control period to 744 microequivalents per minute some 60 to 80 minutes after the diuretic; an increase of about 500 microequivalents per minute.

On the day preceding the second experiment 10 gms. of ammonium chloride were given orally in divided doses. The inherent diuretic action of the salt was largely spent on the succeeding day, for the control rates of sodium excretion averaged but 48 microequivalents per minute.[3] However,

[1] These studies were supported by grants from the National Heart Institute, National Institutes of Health and the Life Insurance Medical Research Fund.

[2] We wish to thank Winthrop-Stearns, Inc., for their generous supply of salyrgan.

[3] The chloride ion, left after the ammonia has been converted into urea by the liver, is neutralized by base derived from bicarbonate and is partially excreted by the kidney as sodium chloride. This loss of sodium chloride and its associated water we consider a consequence of the inherent diuretic action of ammonium chloride. It is relatively short-lived in comparison with the more prolonged disturbance in plasma anion pattern and pH occasioned by the salt.

TABLE I

A comparison of the diuretic responses to salyrgan under conditions of normal acid base balance and acidosis induced by ingestion of ammonium chloride and by inhalation of 6.5 per cent carbon dioxide in air. Dog R

Time	Urine flow	Clearance		Plasma concentration				pH		Rate of excretion			
		Creatinine	PAH	Na	K	Cl	HCO_3	Plasma	Urine	Na	K	Cl	HCO_3
mins.	*cc./min.*	*cc./min.*		*milliEq./liter*						*microEq./min.*			
Salyrgan alone													
−20–0	3.00	80.3	446	149	2.8	118	21.3	7.47	7.12	247	76.6	234	38.4
0	80 mg. Hg as salyrgan												
0–20	3.80	80.7	477	148	3.1	117	21.1	7.44	7.13	362	55.9	326	48.6
20–40	3.45	75.6	423	148	2.4	116	21.0	7.45	6.36	372	40.7	408	8.60
40–60	4.70	68.4	414	148	2.4	115	19.6	7.47	5.81	580	42.9	566	2.60
60–80	6.05	64.6	412	149	3.0	115	20.7	7.46	5.70	744	44.1	815	2.43
80–100	4.65	58.2	370	148	2.9	113	20.8	7.44	5.60	538	41.9	610	1.20
100–120	3.15	53.7	383	147	2.5	113	20.5	7.46	5.57	331	39.7	388	0.72
Salyrgan plus NH_4Cl													
−40–20	1.50	90.5	364	146	3.0	117	15.4	7.36	5.39	60	45.8	133	0.26
−20–0	1.05	72.2	344	144	2.9	115	14.5	7.36	5.52	36	35.6	83	0.27
0	80 mg. Hg as salyrgan												
0–20	3.30	100.7	492	145	2.7	121	14.6	7.37	5.67	349	72.3	445	0.94
20–40	11.40	86.5	405	145	2.8	120	14.7	7.40	5.49	1,510	67.2	1,631	2.01
40–60	10.40	81.9	388	146	2.9	119	13.9	7.42	5.34	1,410	64.5	1,575	1.31
60–80	5.50	61.6	268	146	2.9	118	14.0	7.42	5.21	690	54.5	820	0.53
80–100	3.10	54.8	236	150	2.8	121	14.9	7.41	5.12	320	48.0	434	0.27
100–120	3.00	54.3	227	151	2.9	120	15.3	7.41	5.15	310	45.9	407	0.38
Salyrgan plus CO_2													
−40–20	1.40	78.4	346	149	3.02	115	23.4	7.28	7.46	147	31.8	126	22.6
−20–0	1.60	82.6	328	148	2.87	114	25.3	7.28	7.52	214	36.0	171	42.3
0	80 mg. Hg as salyrgan												
0–20	1.90	82.7	314	149	2.69	113	24.6	7.28	7.56	276	40.6	210	59.0
20–40	4.60	94.3	312	150	2.80	116	23.8	7.30	6.12	689	47.5	723	8.46
40–60	5.70	82.7	268	148	2.91	113	24.6	7.29	5.53	818	48.5	912	2.54
60–80	4.75	77.6	261	149	2.91	111	25.2	7.29	5.40	700	52.3	801	1.60
80–100	3.30	71.8	250	147	2.83	107	25.5	7.29	5.29	481	53.5	589	0.97
100–120	2.80	67.1	242	150	2.90	110	25.5	7.32	5.26	378	53.5	486	0.77

following salyrgan, the rate of sodium excretion increased to 1,510 microequivalents per minute. The absolute increase in rate of sodium excretion after salyrgan was nearly three times that observed in the control experiment.

For 30 minutes preceding the third experiment and throughout its course as well, the animal inhaled 6.5 per cent carbon dioxide in air through a tight fitting respiratory mask and a two-way Douglas valve. Following salyrgan, sodium excretion increased from a control level of roughly 200 to a peak value of 800 microequivalents per minute. The extra sodium excretion thus amounted to some 600 microequivalents per minute, a value comparable with that observed in the control experiment, but far less than that observed in the ammonium chloride experiment.

If one compares the values for plasma pH in

the three experiments, one may rule out any significant contribution of acidity of the body fluids to the enhancement of diuresis which follows ammonium chloride. Thus plasma pH was considerably more depressed by the inhalation of carbon dioxide than by the ingestion af ammonium chloride. Similarly urine acidity does not correlate well with the magnitude of the diuretic response. It is true that urine pH was uniformly low in the ammonium chloride experiment. However, if one compares only the two periods in each experiment in which maximal sodium loss occurred, no convincing correlation between acidity and sodium loss can be established. Thus values of 5.49 and 5.34 were noted in the ammonium chloride experiment, 5.53 and 5.40 in the carbon dioxide experiment, and 5.81 and 5.70 in the control experiment. There remains as the factor of most probable significance [4] in explaining potentiation, the difference in anion pattern of the blood plasma. More specifically we feel that the absolute level of plasma chloride is the significant factor. Thus ammonium chloride increased the plasma chloride level [5] and proportionally reduced the plasma bicarbonate concentration without significantly affecting total base or anion concentration. Marked potentiation resulted. Carbon dioxide inhalation on the other hand increased the bicarbonate level without significantly affecting the chloride level. No potentiation was evident. In our entire series of experiments, including those in which sodium bicarbonate was administered, the magnitude of the diuretic response to a standard dose of salyrgan correlated better with the plasma concentration of chloride than with any other factor. However, experimental conditions were such that plasma sodium concentration did not vary significantly. Hence the correlation in an inverse fashion was essentially as good with plasma bicarbonate.

The results of 20 experiments on dogs R and T performed in a manner identical with those presented in Table I are plotted in Figures 1 to 4. Figures 1 and 2 relate the rate of excretion of sodium in microequivalents per minute to time before and after the intravenous administration

[4] The greater filtration rate observed in the ammonium chloride experiment presented in Table I has not been a consistent finding. Although it must have contributed to the marked potentiation of the action of the mercurial diuretic in this experiment, it has not been observed in others in which significant potentiation occurred.

[5] This animal normally exhibited somewhat higher chloride and lower bicarbonate concentrations in the plasma than does the average animal.

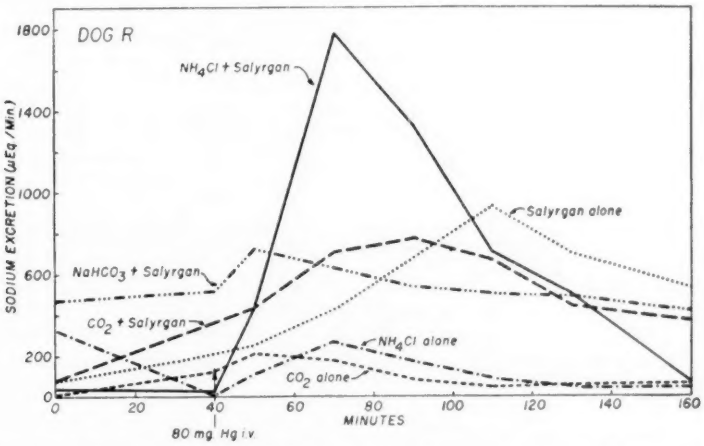

FIG. 1. A COMPARISON OF THE RATES OF SODIUM EXCRETION UNDER A VARIETY OF CONDITIONS IN WHICH PLASMA ANION PATTERN HAS BEEN ALTERED

The curve labeled Carbon Dioxide plus Salyrgan is the average of three experiments. Salyrgan Alone and Salyrgan plus Ammonium Chloride are each the average of two experiments. Each of the other curves represents one experiment.

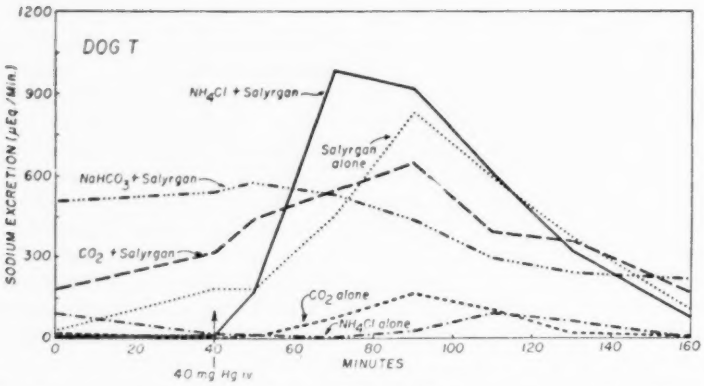

FIG. 2. A COMPARISON OF THE RATES OF SODIUM EXCRETION UNDER A VARIETY OF CONDITIONS IN WHICH PLASMA ANION PATTERN HAS BEEN ALTERED

The curve labeled Salyrgan Alone is the average of three experiments. Carbon Dioxide plus Salyrgan and Ammonium Chloride plus Salyrgan are each the average of two experiments. Each of the other curves represents one experiment.

of salyrgan. Each curve represents the average of one to three similar experiments on one dog, the exact numbers being noted in the legends. The responses to salyrgan alone and to salyrgan during the inhalation of 6 to 8 per cent carbon dioxide are identical within reasonable limits of experimental variability in Dog R (Figure 1). In Dog T (Figure 2), the increase in sodium ex-

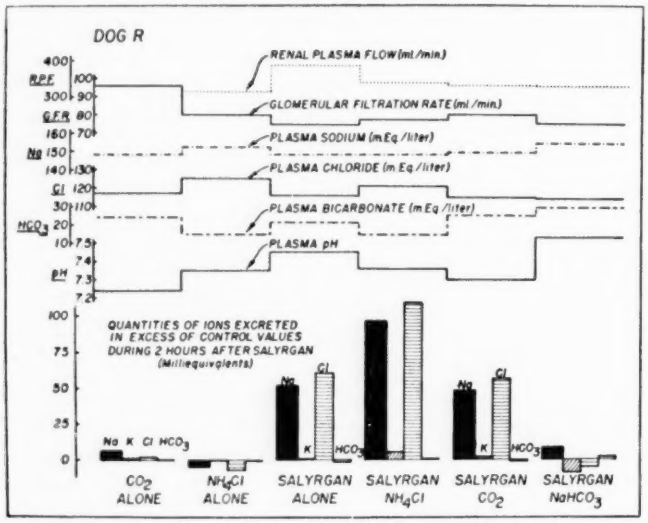

FIG. 3. ELECTROLYTE EXCRETION, PLASMA CONCENTRATIONS, GLOMERULAR FILTRATION RATE, AND RENAL PLASMA FLOW UNDER A VARIETY OF CONDITIONS IN WHICH PLASMA ANION PATTERN HAS BEEN ALTERED

Each block represents total excretion during two hours in excess of the control excretion.

cretion over and above the control level is actually less during carbon dioxide inhalation, despite marked respiratory acidosis.

In Dog R, the response to salyrgan following the administration of ammonium chloride is significantly greater than in control experiments and demonstrates a synergistic type of potentiation of the mercurial by the acidifying agent. In Dog T the synergism is less evident, yet if one considers the magnitude of the increase in sodium excretion over control rates, it is probably real.

The curve designated $NaHCO_3$ plus salyrgan was derived from an experiment in which 15 gms. as controls for the experiments in which salyrgan was given following ammonium chloride and during the inhalation of carbon dioxide.

The data derived from these experiments are summarized in Figures 3 and 4 in such fashion as to permit a comparison of their significant points of difference. In the upper half of each figure are plotted the average values of renal plasma flow, glomerular filtration rate, plasma concentration of sodium, chloride and bicarbonate and plasma pH, noted during the two hours following salyrgan. In the lower half of each figure are shown in block form the milliequivalents of

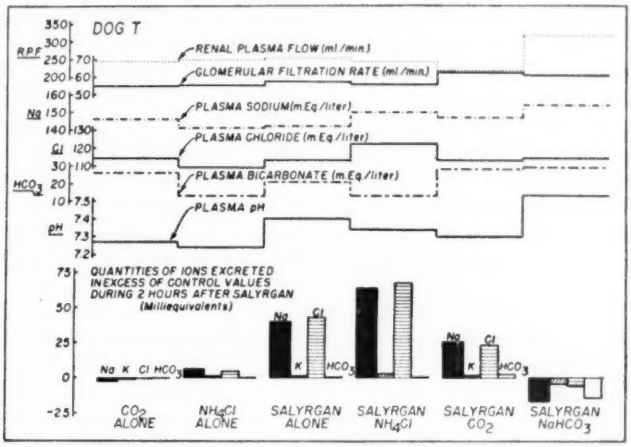

FIG. 4. ELECTROLYTE EXCRETION, PLASMA CONCENTRATIONS, GLOMERULAR FILTRATION RATE, AND RENAL PLASMA FLOW UNDER A VARIETY OF CONDITIONS IN WHICH PLASMA ANION PATTERN HAS BEEN ALTERED

Each block represents total excretion during two hours in excess of the control excretion.

of sodium bicarbonate per day were administered on the three days preceding the experiment and 10 gms. on the day of the experiment. Most of the sodium excreted during the experiment was eliminated as bicarbonate, and the urine was quite alkaline. The plasma level of bicarbonate was elevated and that of chloride depressed. In this experiment and in others like it, salyrgan exhibited little or no diuretic activity, a fact which has been previously noted by other investigators (5, 14). The two curves designated NH_4Cl alone and CO_2 alone were derived from experiments in which no mercury was given, and serve

sodium, potassium, chloride and bicarbonate excreted during the two hours following salyrgan in excess of what would have been excreted had the control rate been maintained. If a block is above the base line, the rate of excretion of that ion was increased by salyrgan; if below the base line, the rate of excretion declined following the diuretic. This mode of expression more accurately describes synergistic potentiation of the diuretic action of salyrgan by ammonium chloride than does the peak rate of excretion presented in Figures 1 and 2. It likewise clearly demonstrates abolition of diuresis by the prior administration

of sodium bicarbonate. It is evident on inspection of the upper part of the chart that of the several variables only two can be correlated with the greater diuretic response to salyrgan following ammonium chloride, namely, reduced plasma bicarbonate and increased plasma chloride concentrations. No causal relationship to increased renal plasma flow and glomerular filtration rate nor to decreased plasma pH can be demonstrated.

DISCUSSION

Experiments presented above rule out acidity of the plasma and interstitial fluid as a cause of potentiation of mercurial diuretics by acidifying salts. Thus inhalation of 6 to 8 per cent carbon dioxide, which induces a more severe acidosis than does the ingestion of 10 gms. of ammonium chloride, has no effect on salyrgan diuresis.

The data are less conclusive with respect to the relationship between urinary acidity and diuretic efficacy of mercurials. There has been in this series of experiments a marked lability of urinary pH under all conditions other than those of ammonium chloride acidosis and sodium bicarbonate alkalosis, a fact which renders difficult any assessment of the significance of the acidity of urine. As noted in the discussion of the three experiments presented in Table I, there is suggestive evidence that urinary pH is not a determining factor.

More conclusive evidence for this view is presented in the two experiments outlined in Table II. In these experiments acidosis was induced by the oral administration of ammonium chloride on the day preceding the experiment. On the experimental day, the animal was anesthetized with chloralose (100 mg. per kg.). Saline containing a small amount of neutral sodium phosphate was infused for 2½ hours prior to and throughout the course of the experiment. After two control periods 160 mg. of mercury as mercuhydrin were administered intravenously. The high rate of infusion of saline protects the animal against the acute circulatory collapse which often follows large doses of mercurial diuretics and also produces a prolonged, sustained mercurial diuresis (15). Phosphate was given to stabilize urine pH and to avoid the disturbing fluctuations often ob-

TABLE II

The effect of an abrupt change in urinary pH during a mercurial diuresis on ion excretion

Time	Urine flow	Plasma concentration			pH		Rate of excretion			Time	Urine flow	Plasma concentration			pH		Rate of excretion		
		Na	Cl	HCO_3	Plasma	Urine	Na	Cl	$BHCO_3$			Na	Cl	HCO_3	Plasma	Urine	Na	Cl	$BHCO_3$
mins.	*cc./min.*	*milliEq./liter*					*microEq./min.*			*mins.*	*cc./min.*	*milliEq./liter*					*microEq./min.*		
−30–15	10.8	149	127	12.9	7.28	5.84	578	410	6.38	−30–15	10.7	158	129	17.2	7.32	5.79	332	335	7.45
−15–0	9.55	149	127	13.1	7.26	5.89	870	730	6.50	−15–0	8.73	156	130	16.4	7.33	5.83	272	252	5.94
160 mg. Hg as mercuhydrin										160 mg. Hg as salyrgan									
0–15	15.7	151	128	12.9	7.29	5.87	1860	1720	10.2	0–15	9.06	160	131			5.78	363	254	5.98
15–30	15.4	151	129	12.1	7.30	5.38	1980	2020	2.62	15–30	6.60	157	132	12.9	7.40	5.03	720	772	.62
30–45	22.9	151	125	—	7.30	5.19	2990	3110	2.29	30–45	13.3	159	136			5.30	1720	1753	1.73
45–60	23.0	151	123	12.1	7.33	5.11	2940	3100	1.68	45–60	19.7	161	133	11.3	7.34	5.51	2620	2680	4.14
										60–75	21.0	161	136			5.54	2800	2860	6.10
Carbonic anhydrase inhibitor, 10 mg./kg.										Carbonic anhydrase inhibitor, 20 mg./kg.									
60–75	24.6	149	123	12.2	7.34	6.28	3310	3270	68.6										
75–90	20.3	149	122	13.7	7.28	6.56	2750	2520	125	75–90	22.5	162	136	13.0	7.33	6.53	3120	2990	93.0
90–105	18.3	151	122	13.8	7.27	6.40	2560	2320	69.4	90–105	22.2	161	135			6.61	3080	2910	110
105–120	17.3	151		14.4	7.26	6.31	2470	2340	52.6	105–120	20.4	164	136	13.7	7.28	6.52	2780	2660	72.8
BAL, 1 cc.										BAL, 1 cc.									
120–135	12.4	149	119	13.3	7.28	6.41	1930	1740	55.7	120–135	15.9	168	137			6.50	2040	1907	58.2
135–150	5.27	150	123	13.6	7.24	6.95	895	453	140	135–150	9.21	167	139	13.0	7.24	6.76	900	683	75.8
150–165	4.69	149	123	13.0	7.24	6.96	740	302	139	150–165	9.57	170	142			6.82	834	616	85.6

served when the urine is poorly buffered. Urine flow and sodium excretion rose rapidly to reach a plateau 30 to 40 minutes after the mercurial. A priming dose of the carbonic anhydrase inhibitor (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide[6] [10 to 20 mg. per kg.]) was given intravenously to shift urine pH rapidly toward an alkaline reaction, and the inhibitor was also infused at a rate of 2 to 4 mg. per minute. Finally, 1 cc. of BAL was given intramuscularly to block the action of the mercurial diuretic. It is evident that a rapid change in urine pH in the absence of any significant change in the composition of the plasma was without effect on the action of the mercurial diuretic. Thus the peak urine flows and peak rates of excretion of sodium and chloride obtained after the mercurial were not depressed following the administration of the inhibitor despite a significant increase in urine pH.

The inherent natriuretic action of the inhibitor is represented by the increased excretion of bicarbonate. Blockage of exchange of hydrogen ion for sodium ion leads to increased excretion of sodium equivalent to the increased excretion of bicarbonate. It is evident from Table II that this quantity constitutes an insignificant fraction of total sodium excretion. BAL, which abolishes the mercurial natriuresis and chloruresis, returns the excretion of sodium and chloride toward control levels. There remains superimposed the inherent natriuretic action of the inhibitor.

In all of our experiments the magnitude of the diuretic response to salyrgan appeared to be directly related to the plasma chloride concentration and inversely related to the plasma bicarbonate concentration. Under the condition of our experiments, no significant relationship exists between plasma sodium concentration and mercurial response. These facts are illustrated in Figures 5 and 6 in which excess sodium excreted during the two hours after salyrgan is plotted against the average concentrations during the same period of plasma chloride and plasma sodium. Each point represents a single experiment such as those presented in Table I. The coefficient of correlation for the 16 pairs of observations using the mean concentration values and the total excess excretion values after administration of salyrgan, shows the correlation between plasma chloride concentration and excess sodium excretion to be $r = +.70$ ($p = .0025$). This is a statistically significant degree of correlation. The correlation between plasma sodium concentration and excess sodium excretion is $r = -.13$ ($p = > .6, < .7$). This is not significantly different from zero correlation. In general an increase in plasma chloride induced by ammonium chloride was associated with an essentially equivalent reduction in plasma bicarbonate. It is, therefore, obvious that if sodium excretion is directly related to the plasma concentration of one of these anions, it must be inversely related to that of the other.

We feel that the relationship to plasma chloride is the significant one, and that if mercury specifically blocks one ion absorptive mechanism, that mechanism is the one for chloride absorption. In-

[6] We wish to thank American Cyanamid Company for their generous supply of the carbonic anhydrase inhibitor.

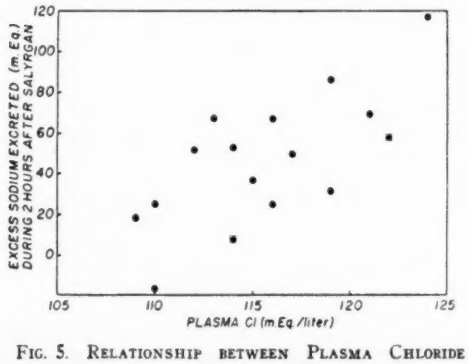

FIG. 5. RELATIONSHIP BETWEEN PLASMA CHLORIDE CONCENTRATION AND THE EFFECTIVENESS OF SALYRGAN IN PRODUCING SODIUM DIURESIS

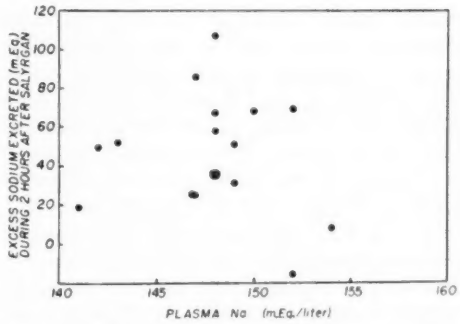

FIG. 6. RELATIONSHIP BETWEEN PLASMA SODIUM CONCENTRATION AND THE EFFECTIVENESS OF SALYRGAN IN PRODUCING SODIUM DIURESIS

creased sodium excretion following mercurial diuretics is thus a more or less passive consequence of increased chloride elimination. This view is based on the following facts: (*a*) After salyrgan the excretion of chloride regularly exceeds that of sodium. This can be seen in Table I and Figures 3 and 4 and has previously been noted by Blumgart and his co-workers (8, 16). Others (2, 4, 17) have correlated the efficacy of a mercurial diuretic with the plasma chloride concentration. (*b*) Bicarbonate excretion is not increased in mercurial diuresis (*cf.* Figures 3 and 4). Were sodium reabsorption specifically blocked, one would anticipate that the excretion of both bicarbonate and chloride would be increased, rather than that of chloride alone. Furthermore, the plasma concentrations of chloride and bicarbonate reflect this discrepant loss of chloride. During the course of a mercurial diuresis there is frequently a fall in plasma chloride and a rise in plasma bicarbonate with no change in plasma sodium concentration (Table I, [6, 8, 14, 18]).

Hypochloremia has been cited as one cause of resistance of patients to mercurial diuretics (14); others have not distinguished between hypochloremia and hyponatremia (2, 9). Elevation of plasma chloride by administration of sodium chloride or ammonium chloride has been shown to restore the response of such individuals to mercury (14, 17, 19). Our results are in agreement with such a view, and extend it by suggesting that potentiation of diuretic action by ammonium chloride is a consequence of the hyperchloremia induced.

Although we have not studied other acidifying agents known to potentiate mercurial diuretics, we would anticipate that a similar explanation could account for their effects. The action of calcium chloride would obviously parallel that of ammonium chloride for both substitute chloride for bicarbonate in the body fluids (20–22). Ammonium nitrate not only increases the plasma concentration of nitrate, but to some degree causes the retention of chloride "at the expense of bicarbonate" (3–5). It is probable that ammonium sulfate produces the same effect (22). Furthermore, it has been shown that when the administration of nitrate causes a fall in plasma chloride, mercurial diuresis is reduced (4). A single explanation could thus account for the action of these several acidifying agents, namely, elevation of plasma chloride level. Since the greatest increase in plasma chloride results from the ingestion of ammonium chloride, it is not surprising that it is the most effective of the potentiating agents and the one which is commonly employed clinically.

SUMMARY

In experiments on normal dogs, it has been observed that the administration of 10 gms. of ammonium chloride on the day preceding salyrgan, significantly potentiates the action of the mercurial diuretic. This potentiation is not a result of increased acidity of the urine and body fluid induced by the acidifying agent. Rather it is a result of an alteration in anion pattern of the body fluids, namely, a substitution of chloride for bicarbonate. It is suggested that mercurial diuretics specifically block chloride absorption and that the potentiation of diuretic action by ammonium chloride is a consequence of the hyperchloremia induced.

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THE RELATIONSHIP OF STREPTOLYSIN S INHIBITOR TO PHOSPHOLIPIDS IN THE SERUM OF HUMAN BEINGS AND EXPERIMENTAL ANIMALS

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INTRODUCTION

The fall in the serum levels of streptolysin S inhibitor (SSI) which usually occurs during the acute phase of rheumatic fever (1, 2) suggested a disturbance of lipoprotein metabolism in this disease. Previous studies indicated that the inhibition of this streptococcal hemolysin by serum is associated with alpha and beta lipoproteins rather than with specific antibody (2–5). Streptolysin S is inhibited by saline suspensions of phospholipids but not by cholesterol or neutral fats (6, 3). In addition, a large proportion of the SSI is destroyed by the action of *Cl. welchii* lecithinase on serum (3). It therefore seemed possible that variation in SSI might reflect a parallel variation of serum lipoproteins rich in phospholipid.

The present study was undertaken to explore the relationship of serum levels of total phospholipid, lecithin and sphingomyelin to the SSI titer in the sera of patients with various diseases associated with disturbances in lipid metabolism, and in the sera of experimental animals with induced hyperlipemia. The observations reported below indicate that subnormal levels of SSI in the serum of human beings are associated with hypophospholipemia while elevation of SSI titer is encountered in diseases characterized by an increased concentration of serum phospholipids. The present study also indicates that an increase in SSI may be induced in animals rendered hyperlipemic by various methods.

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MATERIALS AND METHODS

Clinical material: Sera were obtained from 44 patients with diseases usually associated with either marked elevation or depression of serum phospholipids. The former group included 12 patients suffering from either intrahepatic or extrahepatic biliary obstruction (seven with biliary cirrhosis; three with serum hepatitis; one with a common duct calculus and one with carcinoma of the head of the pancreas); five patients manifesting the nephrotic syndrome; and three patients with hypothyroidism. The group of diseases associated with hypolipemia included seven patients with acute rheumatic fever; five with hyperthyroidism; three with fever of undetermined etiology; two with chronic lymphocytic leukemia; and one case of each of the following conditions: disseminated lupus erythematosus, scleroderma, subacute bacterial endocarditis, tuberculous pleurisy, carcinoma of the thyroid, ulcerative colitis and diffuse bronchiectasis.

Serial bleedings were obtained from several patients in an attempt to demonstrate a progressive rise or fall in phospholipid levels during the course of the disease.

Preservation of sera: Sera obtained from patients and from animals were stored at −20° C. Storage at 4° C. was employed only in instances where titrations and chemical determinations were carried out within 48 hours after blood had been drawn. This precaution was taken because sera stored at 4° C. for longer periods of time may show a progressive rise in SSI titer (3).

Titrations and chemical determinations: Titration of streptolysin S inhibitor was performed by the method of Stollerman and Bernheimer (2). Lipid phosphorus was determined by the method of Fiske and SubbaRow (7) with minor modifications. Phospholipid levels are reported as milligrams of total phospholipid per 100 ml. and were calculated by multiplying the value for lipid phosphorus by 25. Serum phospholipids were partitioned for the determination of lecithin and sphingomyelin by the method of Appleton and associates (8). Lecithin and sphingomyelin are expressed as milligrams per 100 ml. and were calculated by multiplying the choline chloride content of the respective fractions by the factor 5.7. Total and free cholesterol were determined by the method of Schoenheimer and Sperry (9).

Methods for inducing hyperlipemia in rabbits and mice: An elevation of serum phospholipids in rabbits was produced by three different methods:

a) Six rabbits weighing between 1.5 and 2.5 kg. were injected intravenously with the nonionic surface-active agent, Triton A-20 as described by Kellner, Correll, and Ladd (10). Commercial Triton A-20 is a 25% aqueous solution of Triton WR 1339, an alkyl aryl polyether alcohol.[2] A 12.5% solution (Triton A-20 diluted with equal parts of isotonic saline) of this detergent was administered in doses of 1.25 ml. per kg. three times weekly for nine weeks. The rabbits were maintained on a regular diet of Purina rabbit pellets.

b) Ligation of the common bile duct at its entrance into the duodenum was performed under nembutal anesthesia in six rabbits. The animals were bled at approximately weekly intervals thereafter during their survival.

c) Five rabbits were fed cholesterol over a period of two months. Cholesterol was dissolved in ether, mixed with Purina rabbit pellets and ether allowed to evaporate. Cholesterol was consumed at the rate of approximately 19 Gm. per rabbit per week and the animals gained weight steadily throughout the experiment.

Elevation of serum phospholipids in Swiss mice weighing 18–20 Gm. (CFW and Davis and Nagler strains) was induced by intraperitoneal injections of Triton A-20 diluted with equal parts of saline. One tenth ml. was administered three times weekly during the first week and the dose increased to 0.2 ml. thrice weekly thereafter for four weeks. Groups of ten mice were bled from the heart at intervals during the experiment and serum samples of each group were pooled for analysis of SSI, phospholipid and cholesterol levels. The mice were maintained on a diet of Purina Laboratory chow and gained weight steadily during the experiment.

[2] Kindly supplied by Dr. Aaron Kellner and by Rohm & Haas Co.

RESULTS

The relationship of phospholipid levels to SSI in the sera of patients with disturbance of lipid metabolism

Fifty-eight sera obtained from 44 patients were analyzed for SSI and total phospholipid concentrations. Fifty of these sera were further analyzed for lecithin and sphingomyelin levels. The sera were grouped according to their SSI values. Table I shows the mean concentration and the range of values for serum phospholipids at varying levels of SSI.

In general, there appears to be a positive correlation between the concentration of serum phospholipids and the SSI titer. This relationship is best illustrated by the lecithin fraction. It can be noted, however, that the inhibition of streptolysin S by serum is not a simple function of lecithin concentration. At very low levels of serum lecithin the SSI titer falls abruptly while at high lecithin concentrations the rise in SSI tends to level off. It also appears that at normal levels of SSI

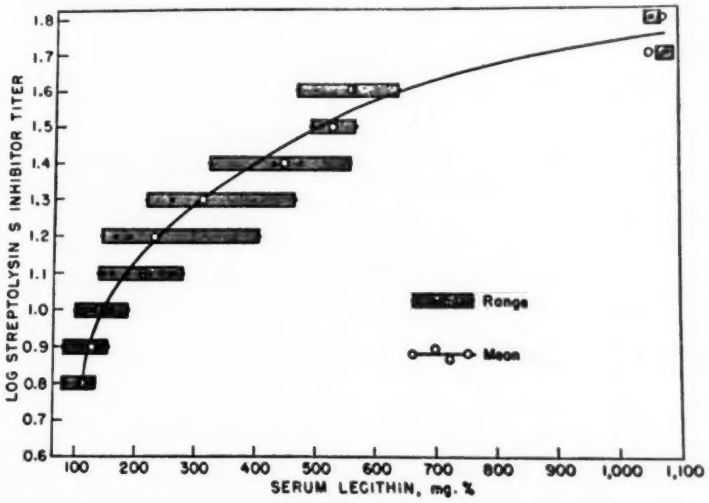

FIG. 1. RELATIONSHIP OF LECITHIN CONCENTRATION TO SSI IN HUMAN SERA
Lecithin values in sera of the same SSI titer.

TABLE I

Concentrations of total phospholipid, lecithin and sphingomyelin in human sera at various levels of streptolysin S inhibition

SSI (u./ml.)	Total phospholipid (mg.%)			Lecithin (mg.%)			Sphingomyelin (mg.%)		
	Mean	Range	No. of sera	Mean	Range	No. of sera	Mean	Range	No. of sera
6.3	176	166–209	5	111	87–123	4	17	9.6–24.4	4
8	185	127–209	7	122	77–154	7	18.8	9.1–29.6	7
10	201	125–264	12	140	108–191	11	25	13.8–40.5	11
12.5	273	179–365	12	212	138–278	10	29.9	13.7–47.2	10
16.7	261	186–425	4	228	143–415	4	27.2	19.2–46.0	4
20	408	302–618	4	315	221–466	3	43.8	21.3–80.0	3
25	585	361–700	7	447	326–560	4	43.1	33.0–49.0	4
33	568	510–625	2	530	490–570	3	56.2	55.4–57.0	2
40	663	520–740	3	558	470–642	3	55.4	37.8–65.0	3
50	1,240	–	1	1,075	–	1	76.4	–	1
62.5	1,275	–	1	1,057	–	1	92.3	–	1

there is a rather wide range of lecithin values (Figure 1).

The normal SSI titer in human serum varies between 10 and 20 units per ml. with a mean value of 14.5 (2). Normal serum lecithin values by the method employed range between 128 and 219 mg. per 100 ml. Twenty-one of 22 sera with SSI values of 10 units or less contained lecithin in concentration below 160 mg. %. The remaining sample of this group contained 10 units of SSI and 191 mg. % of lecithin. All 14 sera with SSI values of 20 units or higher contained lecithin in concentrations greater than 219 mg. %, the upper limit of normal.

The highest levels of SSI were found in two cases of biliary cirrhosis (50 and 62.5 units per ml., respectively). These patients also showed the highest concentrations of lecithin encountered (1075 and 1057 mg. %, respectively). Ten out of 12 patients with biliary obstruction had ele-

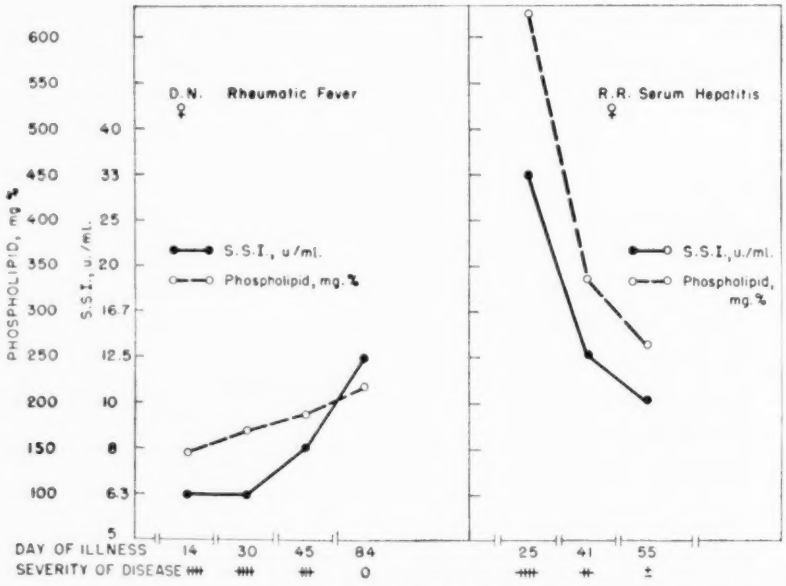

FIG. 2. RELATIONSHIP OF SSI AND PHOSPHOLIPID LEVELS IN SERUM DURING THE COURSE OF ACUTE RHEUMATIC FEVER AND SERUM HEPATITIS

vated SSI titers and these were associated with serum lecithin values greater than 325 mg. per 100 ml. Only one patient with biliary obstruction and elevated serum phospholipids failed to manifest an abnormally high SSI. The remaining patient in this group with obstructive jaundice had a low SSI titer of 10 units per ml., and this was associated with a low serum lecithin value of 143 mg. per 100 ml. This patient had evidence of severe liver damage.

Of the group manifesting nephrotic syndrome, four out of five patients showed a rise in SSI corresponding with an abnormal elevation of serum lecithin.

Seven patients with severe acute rheumatic fever showed SSI values of 10 units or lower. All seven had concentrations of serum lecithin below 160 mg. per 100 ml. (the mean normal value) and in five of the seven the serum lecithin level fell below 128 mg. per 100 ml., the lower limit of normal. A group of eight patients suffering from miscellaneous diseases, in which SSI titers were also found to be low, all showed serum lecithin values below the normal and five of the eight had values of less than 128 mg. per 100 ml.

One patient with rheumatic fever and one with serum hepatitis afforded the opportunity to follow contrasting changes in serum lecithin by serial bleedings during the course of their respective diseases. The patient with rheumatic fever showed low serum lecithin levels during the acute stage of the disease which gradually rose to normal as rheumatic activity subsided. This was accompanied by a similar change in the SSI titer. The patient with serum hepatitis had a markedly elevated serum lecithin level during the acute stage of jaundice. As the disease subsided, lecithin levels returned to normal. This was accompanied by a parallel fall in SSI titer (Figure 2).

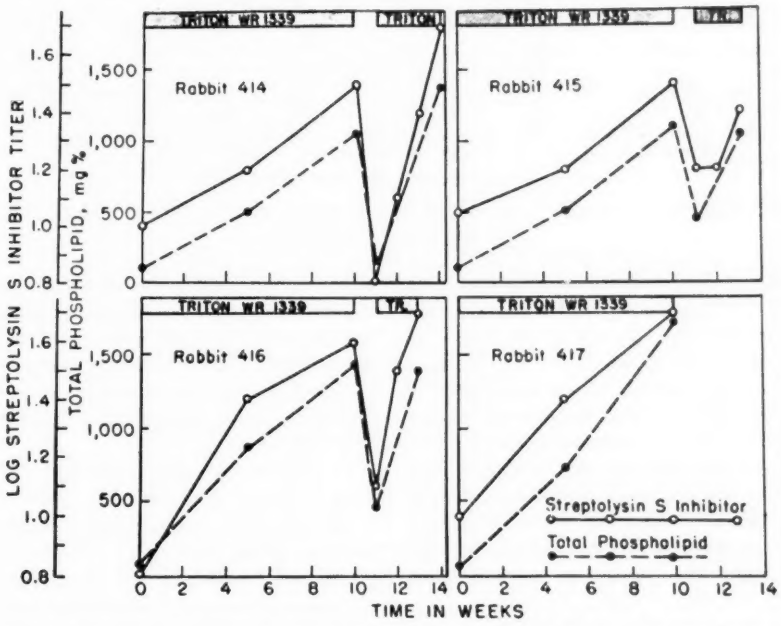

FIG. 3. EFFECTS OF THE INTRAVENOUS ADMINISTRATION OF TRITON WR 1339 ON THE CONCENTRATIONS OF SSI AND TOTAL PHOSPHOLIPID IN RABBIT SERA

The effects of induced hyperphospholipemia on the level of SSI in the serum of rabbits

a) Following injections of Triton A-20: Repeated intravenous injections of Triton A-20 were followed by a rise of both SSI and total phospholipid in the serum of four rabbits. The rise

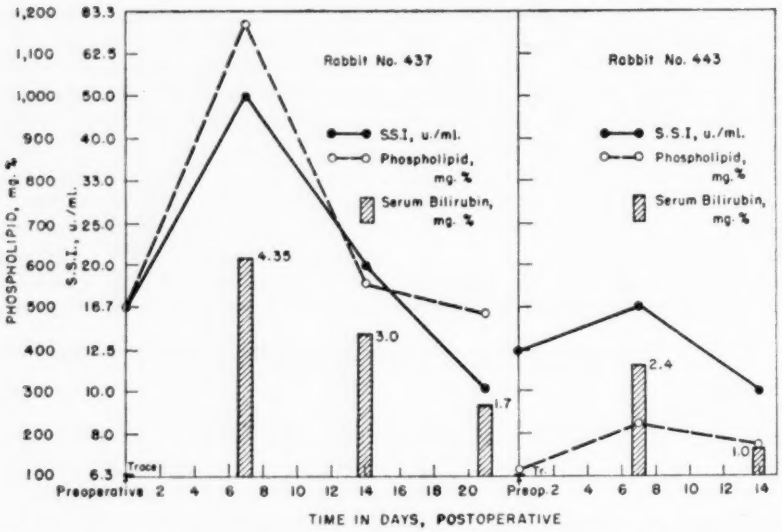

FIG. 4. EFFECT OF LIGATION OF COMMON BILE DUCT ON THE LEVELS OF SSI AND PHOSPHOLIPID IN THE SERUM OF RABBITS

in total phospholipid reached a peak after nine weeks (27 injections) in accordance with the phospholipid response previously described by Kellner, Correll, and Ladd (10). The level of SSI also rose progressively to a peak value of 50 units at the height of hyperlipemia. A significant rise in SSI was not apparent, however, until phospholipid values had increased to several times normal levels. When injections of Triton A-20 were discontinued, both total phospholipid and SSI levels returned to normal values and rose promptly again when injections were resumed (Figure 3).

Triton A-20 was itself found to inhibit streptolysin S *in vitro*. However, when the detergent was added to serum this inhibition was not apparent until concentrations of Triton A-20 exceeding 2% were reached. Although it is improbable that such concentrations of Triton A-20 were ever achieved *in vivo* with the dosage employed, other methods for the elevation of serum phospholipid were also employed to obviate this possible objection to the significance of the above data.

b) Following ligation of the common bile duct: The effect of ligation of the common bile duct on the concentrations of SSI and phospholipids of the serum of four rabbits was observed. Two of the rabbits developed marked hyperphospholipemia and this was associated with a comparable elevation of SSI. The remaining two animals developed only a mild rise in serum phospholipid and showed, correspondingly, but a slight increase in SSI (Figure 4).

c) Following cholesterol feeding: Feeding cholesterol to four rabbits was also followed by a rise in SSI and serum phospholipids (Table II). As with the above methods of inducing hyperphospholipemia, the titer of SSI followed variation in phospholipid concentration. Determination of free and total cholesterol was also performed on these sera since it has been observed that there is an increase in the ratio of serum cholesterol to phospholipid in rabbits fed cholesterol, while in rabbits receiving Triton A-20 this ratio may be reversed (10, 11). Despite the great increase in serum cholesterol observed in these rabbits, the SSI titer was no greater than that found in Triton A-20 treated rabbits at comparable concentrations of serum phospholipid. Therefore it appears that cholesterol levels have little direct influence on the concentration of SSI, an observation which was expected, since saline suspensions of cholesterol fail to inhibit streptolysin S.

TABLE II

The behavior of SSI in relation to phospholipid and cholesterol in the serum of cholesteral-fed rabbits

No. weeks on chol. diet*†	Rabbit No. 409				Rabbit No. 410				Rabbit No. 431				Rabbit No. 433				Rabbit No. 436			
	SSI (u./ml.)	Phos. (mg. %)	Chol. (mg. %)		SSI (u./ml.)	Phos. (mg. %)	Chol. (mg. %)		SSI (u./ml.)	Phos. (mg. %)	Chol. (mg. %)		SSI (u./ml.)	Phos. (mg. %)	Chol. (mg. %)		SSI (u./ml.)	Phos. (mg. %)	Chol. (mg. %)	
			Free	Total			Free	Total			Free	Total			Free	Total			Free	Total
0	12.5	101	33.5	100	20	165	47	213	12.5	167	49	142	10	87	29	104	12.5	139	47	137.5
4	40	932	870	2,920	50	730	594	2,320	40	800	630	2,050	33	646	496	1,810	50	984	872	3,200
8	40	724	651	2,480	40	892	860	2,840	25	606	476	1,740	33	650	594	1,820	50	1,410	1,216	4,450
10	16.7	300	288	821	16.7	455	344	1,241	12.5	316	214	728	16.7	279	212	706	40	1,136	944	2,860

* Rabbits consumed about 19 Gm. of cholesterol per rabbit per week.
† Cholesterol discontinued and normal diet resumed after eight weeks.

The effect of intraperitoneal injections on the concentrations of SSI and phospholipid in the serum of mice

A rise in serum phospholipid may be induced in mice, as well as in rabbits, by the administration of Triton A-20 and here, too, hyperphospholipemia is associated with a rise in SSI titer (Table III). The mouse sera, like rabbit sera, became intensely milky as the blood lipids rose. The mice appeared healthy and gained weight during the administration of the detergent.

TABLE III

SSI and phospholipid levels in the serum of mice following repeated intraperitoneal injections of Triton A-20

No. of injections*	Date bled†	SSI (u./ml.)	Phospholipid (mg. %)
0	1/4/51	25	182
15	2/7/51	40	278
25	3/2/51	62.5	2,000

* Triton A-20 injected as a 12½% solution of Triton WR 1339 in volumes of 0.2 ml. intraperitoneally three times weekly.

† On the indicated dates 10 mice were sacrificed by bleeding from the heart, the blood samples pooled and determinations of SSI and phospholipid performed on the serum thus obtained.

DISCUSSION

It is apparent that variations in the titer of SSI reflect, in general, similar variations in serum phospholipid concentration. The subnormal levels of SSI encountered in rheumatic fever, and in certain other diseases, are indicative of hypophospholipemia. Conversely, elevations in SSI above normal levels have been found only in hyperphospholipemic sera.

A considerable range of phospholipid concentration may, however, occur at any given titer of SSI and at very high serum concentrations of phospholipid the increase in SSI tends to level off (Figure 1). This indicates that the inhibition of streptolysin S by serum is not a simple function of phospholipid concentration. Since the phospholipids of serum probably exist as components of lipoprotein molecules (12, 13), it is possible that the SSI titer is affected by variations in the physico-chemical composition of these proteins which occur in hyperlipemic states (13).

The significance of the fall in serum phospholipid which occurs in rheumatic fever is not un-

derstood. The concentration of serum cholesterol has also been observed to fall during the acute phase of this disease (14), and to be lower in patients with rheumatic heart disease than in patients with hypertensive or arteriosclerotic heart disease (15). Both lipids decrease in concentration in the serum of patients with a variety of acute and chronic infectious diseases (16, 17). It has been suggested that the fall in the concentration of serum lipids is not a feature of a particular disease process but rather a non-specific metabolic disturbance common to debilitation and malnutrition (18). While this view appears reasonable, it has not yet been demonstrated that the hypolipemia of infectious diseases is induced by either hyperpyrexia or dietary deficiency alone (15).

The behavior of lipoproteins in hypolipemic states has not yet been adequately studied. Whether the newer methods for the separation and analysis of alpha and beta lipoproteins (12, 19, 20) will reveal specific differences in the composition of these proteins during hypolipemia remains to be determined.

The non-antigenic properties of streptolysin S have not heretofore provided the opportunity to study the pathogenicity of this toxin during streptococcal infections by classical antibody protection techniques. The induction of hyperlipemia in rabbits and mice affords a method of increasing SSI in these animals and thus potentially offers a unique opportunity to evaluate the role that a normal inhibitor may play as a host defense against a bacterial toxin. The increase in SSI titer is, however, clearly not of the magnitude usually encountered with the appearance of specific antibodies in serum. Studies are in progress to determine whether hyperlipemic animals develop increased resistance to the toxic effects of streptolysin S and to streptococcal infection.

SUMMARY

1. Variations in streptolysin S inhibitor titer are associated with similar variations in the phospholipid content of human sera.

2. Elevated serum levels of SSI are found only in diseases associated with hyperphospholipemia. Conversely, a fall in SSI titer occurs in acute rheumatic fever and other diseases when serum phospholipids fall below normal levels.

3. The induction of hyperphospholipemia in rabbits by repeated intravenous injections of the nonionic detergent, Triton A-20, by ligation of the common bile duct, and by feeding cholesterol results in an elevation of SSI titer. The SSI titer in mice may also be elevated by repeated intraperitoneal injections of Triton A-20.

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THE ALVEOLAR-ARTERIAL OXYGEN PRESSURE GRADIENT IN ANEMIA [1]

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INTRODUCTION

In anemia the oxygen-carrying capacity of the blood is reduced but it is generally believed that the resting arterial oxygen saturation is normal (1–5). Because of the characteristics of the oxyhemoglobin dissociation curve, an appreciable degree of reduction in arterial oxygen tension below the normal level might be present and not be detected by measuring the per cent of arterial oxygen saturation from oxygen contents of arterial and saturated bloods. Within recent years more sensitive techniques have been developed for measuring arterial pO_2. It is possible to measure the arterial oxygen tension directly and to estimate the difference between this and the alveolar oxygen tension, the so-called alveolar-arterial oxygen tension gradient (6, 7). The demonstration that breathing under certain conditions is significantly influenced by changes in oxygen tension which are difficult to estimate by the older methods of measuring per cent arterial oxygen saturation has stimulated our interest in the influence of arterial oxygenation on respiration in disease. The present report deals with the application of these more sensitive techniques to the problem of arterial oxygenation in anemia.

METHODS

Observations were made on 10 anemic (Hgb. 2.0 – 8.9 grams %) and 11 control subjects (Hgb. 10.5 – 14.9 grams %). There was no evidence of pulmonary disease or congestive heart failure in any subject, as judged by history and by physical and roentgen examination. Five of the control subjects (K. W., V. P., S. V., G. S., and W. A.) were medical students; the remainder were hospital patients.

Expired air was collected in Douglas bags. Several minutes were allowed to accustom the subject to the respiratory apparatus, after which two collections of approximately three minutes each were made. The first of these served as a check to insure that minute volume and respiratory quotient were not fluctuating widely. During the second collection, arterial blood was slowly drawn over a one to two minute period through an inlying needle previously placed in the brachial artery. Where a reduced oxygen mixture was inspired (14.7% O_2, 85.3% N_2), the subject breathed this mixture for at least 15 minutes before the final air collection was begun. Procedures were carried out at an ambient temperature of 23 ± 1.5° C. and a barometric pressure of 748 ± 5 mm. Hg.

"Direct" blood O_2 and CO_2 tensions were measured by the method of Riley, Proemmel, and Franke (6), using some of the modifications suggested by Lambertsen and Bunce (8). The accuracy of this method in our hands was checked against blood which had been brought to known gas tensions by equilibration in a tonometer at 37° C. There were 10 such samples, with hemoglobin contents ranging from 2.0 to 15.0 grams %, CO_2 tensions from 34 to 82 mm. Hg, and O_2 tensions from 55 to 106 mm. Hg. The mean deviations of measured tensions from actual tensions were, for CO_2, -1.8 ± 0.9 mm. Hg (S.D. ± 2.8 mm. Hg); and, for O_2, 0.2 ± 0.7 mm. Hg (S.D. ± 2.3 mm. Hg). "Indirect" CO_2 tensions were also measured in all subjects by methods previously described (9). The mean "indirect" CO_2 tension exceeded the "direct" tension by 1.3 ± 0.9 mm. Hg. (S.D. ± 4.5 mm. Hg). The blood CO_2 tensions reported in Tables I and II represent an average between "direct" and "indirect" determinations. Arterial blood oxygen saturation was determined by a spectrophotometric method (10). "Indirect" O_2 tensions, obtained by referring per cent saturation values to standard oxyhemoglobin dissociation curves, agreed quite satisfactorily with "direct" O_2 tensions in subjects breathing low oxygen mixtures (mean algebraic difference, "direct" minus "indirect" pO_2: -1.0 mm. Hg; mean absolute difference: 5 mm. Hg).

"Ideal" alveolar air was determined by the graphic method of Riley and Cournand (7). Alveolar O_2 tensions by this method agreed quite closely with tensions calculated by the "alveolar" equation (11) during the breathing of both air and the reduced oxygen mixture in all cases, and no substantial difference would result, in these subjects, from the use of the latter expression instead of the former.

RESULTS

The pertinent data from the control group are presented in Table I and that from the group of

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TABLE I

Alveolar and arterial gas tensions in control subjects

Subject	Hgb. (*Gms. %*)	"Ideal" alveolar O_2 tension (*mm. Hg*)	Arterial O_2 tension (*mm. Hg*)	Arterial CO_2 tension (*mm. Hg*)	Alveolar-arterial O_2 tension gradient (*mm. Hg*)
K. W., 23 M	13.6	98.2	104.0	38.9	−5.8
V. P., 27 M	13.7	98.6	98.0	39.0	0.6
D. C., 47 M	13.8	89.2	95.0	43.6	−5.8
S. H., 57 F	11.4	104.1	95.1	32.4	9.0
S. V., 23 M	13.7	99.6	98.7	41.1	0.9
G. S., 23 M	12.8	100.1	96.3	42.9	3.8
W. A., 25 M	12.6	104.8	97.5	35.0	7.3
P. D., 42 F	13.9	100.0	98.0	39.4	2.0
J. H., 39 M	13.9	104.0	94.7	35.2	9.3
W. P., 53 M	11.7	92.0	76.7	38.8	15.3
L. G., 62 F	10.5	90.3	81.0	48.2	9.3
Mean	12.9 ± 0.4	98.3 ± 1.7	94.1 ± 2.4	39.5 ± 1.3	4.2 ± 2.0

anemic subjects in Table II. The mean difference between alveolar and arterial oxygen tensions (A-A gradient) is 4.2 ± 2.0 mm. Hg for the control group and 20.5 ± 4.6 mm. Hg for the anemic group. The difference is quite significant ($p < .01$). The alveolar oxygen tension is slightly higher and the CO_2 tension slightly lower in the anemic than in the control group, but the differences are not significant. A significant difference ($p < .01$) does exist, however, between the arterial oxygen tension of 94.1 ± 2.4 mm. Hg in the control group and that of 82.1 ± 2.6 mm. Hg in the anemic group.

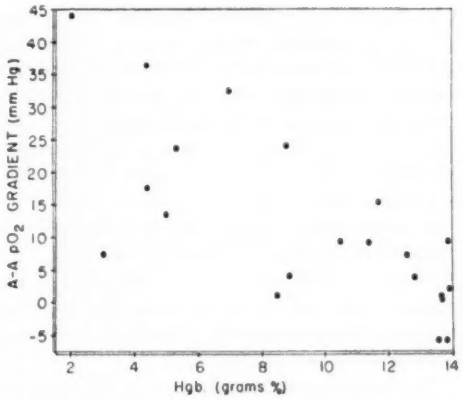

FIG. 1. RELATION BETWEEN HEMOGLOBIN AND A-A pO_2 GRADIENT IN 21 SUBJECTS

A negative correlation exists ($r = -.728$).

TABLE II

Alveolar and arterial gas tensions in anemic subjects

Subject	Comment	Hgb. (*Gms. %*)		"Ideal" alveolar O_2 tension (*mm. Hg*)		Arterial O_2 tension (*mm. Hg*)		Arterial CO_2 tension (*mm. Hg*)		Alveolar-arterial O_2 tension gradient (*mm. Hg*)	
		Air	14.7 % O_2	Air	14.7 % O_2	Air	14.7 % O_2	Air	14.7 % O_2	Air	14.7 % O_2
R. A., 37 F	Pernicious anemia	8.8		110.5		86.4		33.2		24.1	
J. C., 52 M	Chronic blood loss	3.0	2.8	87.2	51.6	79.7	53.9	42.9	39.3	7.5	−2.3
J. C., 38 M	Pernicious anemia	5.3	5.2	108.5	77.0	84.7	50.8	29.4	28.5	23.8	26.2
E. H., 57 F	Chronic blood loss	2.0		117.7		73.5		24.5		44.2	
S. P., 17 F	Chronic blood loss	8.9	8.9	97.7	52.8	93.7	55.9	40.0	37.7	4.0	−3.1
W. P.,* 53 M	Chronic blood loss	4.4	4.4	94.8	47.7	77.2	48.2	42.7	42.5	17.6	−0.5
A. P., 48 M	Multiple myeloma	5.0		95.6		82.1		45.4		13.5	
E. R., 62 F	Chronic blood loss	7.0	7.4	110.5	59.8	78.0	58.8	30.1	31.0	32.5	1.0
L. R., 38 F	Macrocytic anemia	8.5	8.7	96.4	54.5	95.3	58.5	44.0	41.8	1.1	−4.0
S. T., 50 F	Chronic blood loss	4.4	4.2	106.5	54.7	70.1	49.2	40.8	39.9	36.4	5.5
Mean		5.7 ± 0.8		102.5 ± 3.0	56.9 ± 3.6	82.1 ± 2.6	53.6 ± 1.6	37.3 ± 2.3	37.2 ± 2.0	20.5 ± 4.6	3.3 ± 4.0

* Same patient as W. P. in Table I.

In Figure 1 the relation between A-A gradient and hemoglobin concentration is plotted for all subjects of both groups. There is an apparent negative correlation between the two measurements which proves to be significant ($r = -.728$, $p < .01$). The figure indicates a fairly marked tendency for the gradient to scatter more as the hemoglobin concentration is reduced.

In seven anemic subjects the A-A gradient was measured during the inhalation of 14.7% O_2. In all cases but one there was a reduction of the gradient. The mean gradient on the low O_2 mixture was 3.3 ± 4.0 mm. Hg. This is a significant decrease (p approximately .02).

The occurrence of small negative gradients on the low O_2 mixture and in the control group on air is evidently owing to experimental error. Judged by the validation procedure described in the preceding section, the error is small, and it should apply equally to both anemic and control groups.

DISCUSSION

Despite equivalent alveolar ventilation, the arterial blood O_2 tension is significantly lower in the anemic than in the control groups, and the A-A gradient is significantly greater in the anemic group. There is some evidence which bears on the cause of this. Calculations based on principles outlined by Bohr (12) indicate that anemia *per se* should not adversely affect the attainment of equilibrium between the oxygen tensions of alveolar air and pulmonary capillary blood. Lilienthal, Riley, Proemmel, and Franke (13) have pointed out that inhalation of a low oxygen mixture will increase a gradient which depends upon impairment to oxygen diffusion across the alveolar-capillary "membrane," and will decrease a gradient which depends upon admixture of venous blood with oxygenated blood. The effect of a low oxygen mixture in the anemic group suggests that the increased gradient in this case results from venous admixture, rather than impairment of alveolar-capillary diffusion, such as might be caused by pulmonary edema.

Venous admixture to oxygenated blood may occur through such channels as Thebesian veins and bronchial veins which connect with pulmonary veins, or may result from blood flow through imperfectly aerated regions of the lung (13, 14). It also seems possible that some oxygen is lost from blood in the left ventricular cavity by diffusion across the endocardium into the left ventricular muscle. A very large difference in O_2 tension normally exists between arterial blood and heart muscle, and the O_2 tension of ventricular muscle is even lower than normal in anemia (15).

In anemia the per cent oxygen saturation of mixed venous blood is usually decreased (16). The effect of venous admixture in lowering arterial oxygen tension becomes greater as the per cent oxygen saturation of the venous blood is reduced. The question arises as to whether the increased A-A gradient of the anemic subject can be accounted for on the basis of admixture of a normal proportion of venous blood. Enough information exists to allow an estimate. The per cent of venous admixture can be expressed as the ratio $\frac{X - A}{X - V}$, where X is the blood oxygen content corresponding to the oxygen tension of alveolar air, A is the arterial content, and V is the venous content (7). In normal subjects V can be estimated by using an arbitrary $A - V\ O_2$ difference of 4.3 vol. %, without much risk to the calculation. With the present anemic subjects, an $A - V$ difference was assigned to each subject according to hemoglobin concentration, using a regression line calculated from the data of Brannon, Merrill, Warren, and Stead (16). For uniformity, oxygen contents of "pulmonary capillary blood" ("X") and arterial blood were estimated from the corresponding oxygen tensions, using standard dissociation curves, and taking due account of the presence of dissolved oxygen. The result of these calculations in the anemic group is a mean venous admixture of $6.6 \pm 1.4\%$ of the total cardiac output. This is in the same order of magnitude as the value of 4.2% found by Riley and Cournand for eight normal subjects, but is about three times the proportion found in our control group. The data on which the proportions of venous admixture are estimated are not sufficiently exact in this case to allow more than the conclusion that the A-A gradient of anemia can depend upon "venous admixture" in normal or near-normal quantities.

If the per cent oxygen saturation of venous blood were still further reduced, the A-A gradient would be further increased. Himwich and Loebel

(2) found a decrease in the arterial blood oxygen saturation of three anemic subjects during exercise. The methods used were stated to be susceptible to considerable error in anemic subjects, but the present results provide inferential support for this finding. Such a fall in arterial oxygen tension during exercise might contribute significantly to the exertional dyspnea which characterizes anemia (3, 8). It is not easy to account for this dyspnea on the basis of decrease in maximum breathing capacity or increase in breathing requirement by demonstrated stimuli acting along well-recognized pathways. The subject deserves further attention.

It has been suggested that a deficiency of carbonic anhydrase in anemic subjects might interfere with elimination of carbon dioxide in the lungs (3). In the present subjects, who were at rest, the arterial carbon dioxide tension was within the normal range, but the possibility remains that such an effect might become evident during exercise, when the rate of excretion of carbon dioxide is increased.

SUMMARY AND CONCLUSIONS

1. The arterial oxygen tension and alveolar-arterial oxygen tension gradient have been measured in 10 anemic and 11 normal subjects.

2. The anemic group had a significantly lower arterial oxygen tension and higher A-A gradient than the normal group. Taking all subjects together, there was a significant negative correlation between hemoglobin concentration and A-A gradient.

3. It appears possible to account for this effect on the basis of "venous admixture" in normal or near-normal quantities.

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THE INCORPORATION OF C^{14} GLYCINE INTO PROTEIN BY HUMAN LIVER SLICES[1]

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Present knowledge of protein synthesis and indeed of intermediary metabolism in general stems from extensive work in many laboratories using a variety of species. Information concerning human intermediary metabolism has not kept pace with the experimental animal work for obvious reasons. Known species differences make it essential to study humans, either to corroborate the findings in experimental animals or to delineate the areas of difference. While recent reports (1, 2) have indicated that tracer doses of C^{14} compounds may be administered to humans with apparent safety, nevertheless their usefulness at the present time seems limited because of the unknown factors involved and the long half-life.

The *in vitro* techniques with human tissue offer a means of obviating the radiation hazard and of exploiting the value of the C^{14} tool. Biochemical studies of surviving human tissues are few. There are reports on respiration of brain (3, 4) and skin (5), on the cytochrome oxidase system of a variety of normal and malignant tissues (6, 7), on the fate of testosterone in liver (8), and on the incorporation of P^{32} into human leucocytes (9). Recently Tagnon, Schulman, Brunschwig and Lieberman reported on the action of human liver slices on alpha-estradiol (10).

It is the purpose of this report to show that biopsy samples of human liver are capable of being handled in the same manner as tissue from experimental animals, and more specifically to present data on the incorporation of carboxyl C^{14} labeled glycine into the protein by liver slices.

MATERIAL AND METHODS

Biopsies of the liver were obtained during laparotomies which were performed upon patients on the Surgical Service of the Multnomah County Hospital. An attempt was made to obtain specimens from patients whose livers were presumed to be normal. A wedge of tissue, approximately 1.0 gm., was removed and immediately placed into chilled buffer (0° C.). It was rapidly carried to the laboratory and sliced by hand with a razor blade, maintaining the temperature as close to 0° C. as possible. The slices were blotted with filter paper and weighed rapidly. Carboxyl labeled glycine[2] (0.33 microcurie) and 2 cc. of buffer were added to 75–150 mg. of tissue in a 50 cc. glass-stoppered Erlenmeyer flask. The flasks were shaken for four hours at 37° C. in an atmosphere of 95% oxygen and 5% CO_2. The incubation was terminated by precipitating the protein with boiling 1 M. acetate (pH 4.5), and the flasks were placed in a boiling water bath for 10 minutes. The contents of the flasks were transferred to 12 cc. heavy-walled centrifuge tubes and the precipitated protein separated by centrifugation at 2,000–2,500 r.p.m. for three to five minutes. The residue was homogenized with 1 M. acetate in a Potter-Elvehjem glass homogenizer, and the precipitate again separated by centrifugation. The protein was washed six times with 8–10 cc. aliquots of cold 1 M. acetate in the centrifuge tube. Non-radioactive glycine was added to one of the washes to help remove unincorporated labeled glycine by dilution of the isotope. The protein residue was then extracted two times with 3:1 alcohol-ether at temperature of 55° to 60° for three to five minutes and once with ether. The dried protein was suspended in 3:1 petroleum ether-acetone with the aid of the glass homogenizer and poured on an aluminum disc which was enclosed between the inverted upper half of a 3.0 cm. diameter one-ounce jar and its screw top (11). The radioactivity was then counted after drying in air. As controls, tissues were precipitated in zero time in the presence of labeled glycine and otherwise treated as were the experimental samples. Such controls were performed in each experiment. Duplicate flasks were used for each set of conditions, although in later experiments a single zero time control was used.

The buffers used were as follows: *1*) 0.9% KCl and 0.4% $KHCO_3$ saturated with 95% O_2 – 5% CO_2, pH 7.5, and *2*) Krebs-Henseleit buffer containing NaCl 0.72%, $NaHCO_3$ 0.25%, KH_2PO_4 0.10%, $MgSO_4 - 7\ H_2O$ 0.06%, glucose 0.02%, Na citrate 0.03%, and $CaCl_2$ 0.03%. (The $CaCl_2$ is made up in a 1% solution and 0.3 cc. added to 10 cc. of the remainder of the mixture just before using. Otherwise a precipitate forms on standing.) The solu-

[1] This investigation was supported by a research grant from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

[2] Glycine, 0.5 millicurie per millimole, purchased from Tracerlab, Inc., Boston, Mass.

tion, prior to adding the calcium, was saturated with a 95% O_2 – 5% CO_2 mixture.

Radioactivity was expressed as specific activities, *i.e.*, the counts per minute per milligram dry weight of protein, after appropriate corrections for background and self-absorption, the latter being done by means of an empirical correction curve obtained under experimental conditions (12). All counts were made with a flow gas counter. Values are expressed as the means of duplicate pairs.

RESULTS

Table I presents a summary of the clinical data, microscopic evaluation of biopsies, and the experimental observations. Microscopic evaluation of the tissue of all the patients was normal except for J. R. in whom fatty infiltration was present. Liver function tests were not performed for the most part since liver disease was not suspected. Correlations of this type are planned for a future communication. J. R. and H. H. were the only patients who were in poor nutritional condition, having lost approximately 30 and 70 pounds, respectively, at the time of surgery.

It was obviously impossible to control the duration of anaesthesia prior to the biopsy, so that this

TABLE I

Patient	Age	Remarks	Biopsy microscopic evaluation	Protein—*counts per minute per milligram*						
				A	B	C	D	E	F	G
				KCl-$KHCO^3$	Complete Krebs-Henseleit	Krebs-Henseleit without calcium	Krebs-Henseleit without magnesium	Krebs-Henseleit without Ca or Mg	Nitrogen	Cyanide .005 M.
C. A.	77	Cholecystectomy, hydrops	Normal	11.9					13.1 (0)*	
C. S.	61	Ulcer, gastric resection	Normal	7.0						
H. J.	54	Ulcer, gastric resection not done	Normal	8.0					8.1 (0)	
J. R.	74	Carcinoma of stomach with metastases	Fatty infiltration	6.4					7.4 (0)	
A. G.	43	Ulcer, gastric resection	Normal	30.	280					
R. S.	47	Cholecystitis, cholecystectomy	Normal		166	73 (66)			13 (82)	
A. B.	48	Ulcer, gastric resection	Normal		630	250 (60)			23 (96)	
G. N.	67	Cholecystectomy, common duct exploration	Normal		703	480 (32)		203 (71)		
E. H.	66	Cholecystectomy, common duct exploration	Normal		455	236 (48)				50 (89)
R. B.	41	Ulcer, gastric resection	Mild subacute cholangitis		613	206 (66)	350 (43)	165 (73)		38† (94)
C. R.	69	Cholecystectomy, stones	Normal		498		138 (72)			
J. B.	62	Common duct exploration	Normal		539		207 (60)		72† (87)	
H. H.	48	Carcinoma of stomach, inoperable	Normal		240		106 (66)			
C. D.	42	Ulcer, gastric resection	Normal		160		49 (69)	65 (59)		
F. P.	42	Cholecystectomy, hydrops	Normal		396				38 (90)	
		Zero time mean ± S.E.		2.1±0.3	8.7±2.3					
		Mean ± S.E.			425±58	249±59	170±52			

* All figures in parentheses represent % inhibition.

† Single observation. All others are averages of duplicates.

Using as an example the patient C. R., the rate of incorporation of labeled glycine is calculated to be 7.3×10^{-3} mM per gm. of protein per hour starting with a solution containing 6.65×10^{-4} mM glycine and 95 mg. wet weight of liver slices.

TABLE II

Paired experiments	Mean difference*	Number of paired observations	S.E.	t	P
Difference between complete buffer and buffer without calcium	264	5	± 65	4.1	<.025
Difference between complete buffer and buffer without magnesium	235	5	± 53	4.4	<.025
Difference between complete buffer and buffer without either calcium or magnesium	350	3	±122	2.9	>.025

* This is the mean of the five differences of paired specific activities, *e.g.*, Column B minus Column C (Table I) for each of the five patients.

interval varied from 30 minutes after onset, to five hours (in the case of C. D.). All biopsies, except for C. D., were obtained within three hours from the beginning of anaesthesia.

The data presented show that there is a very significant uptake of the radioactive glycine into the liver protein, and that precipitating the protein at zero time in the presence of the radioactive glycine invariably yielded protein with negligible specific activities. It is apparent that the use of a complete Krebs-Henseleit buffer (Column B) resulted in maximum specific activities (160–703). The use of the KCl-$KHCO_3$ buffer (Column A), which had no added calcium, magnesium, citrate, or glucose, yielded protein with specific activities ranging from 7 to 30. Five pairs of observations comparing the full Krebs-Henseleit buffer with the same medium, except that calcium had been omitted (Columns B and C), revealed a consistently lower uptake with the latter solution, the difference being significant, as shown in Table II. It should be noted that these pairs of observations compare tissues from a given patient under the two different conditions. A similar, significant difference was found when magnesium sulfate was omitted from the buffer (Columns B and D). When both calcium and magnesium were omitted (Column E), the percentage inhibition seemed to be even greater, except in the case of C. D. This difference is not significant, however, probably because of the fewer observations. A nitrogen atmosphere had no evident depressing effect on the uptake when the KCl-$KHCO_3$ buffer was used, and a marked inhibiting effect in the presence of the more complete medium (Column F). Cyanide (.005 M.) was an effective inhibitor (Column G).

The figures presented are average values for duplicate pairs of observations for each set of experimental conditions except as noted. The variation of each observation from the mean of its pair of duplicates was less than 15% in 21 out of 30 pairs, and less than 25% in 28 out of 32 pairs.

Data similar to the above have also been obtained using sigmoid colon slices from surgical patients. The specific activities of the protein ranged from 215 to 1,175 under the same experimental conditions. There was apparently the same difference in uptake with buffer variations except that the presence or absence of calcium seemed to have no effect. These observations will be presented in detail at a later date.

DISCUSSION

There have been numerous reports on studies of this type with tissues from experimental animals (11, 13–16). Since the classic work of Schoenheimer (17) the lability of tissue protein and its existence in dynamic equilibrium with its component parts has become an accepted concept and the incorporation of labeled amino acids into protein of adult tissues is an accepted fact. With respect to the relationship between incorporation and protein synthesis there has been considerable discussion among biochemists (18). When an *in vivo* technique is used, there is little doubt that the level of incorporation is a measure of synthesis. When tissue slices and homogenates have been used, however, some doubt has been expressed as to the meaning of "incorporation." However, the weight of existing evidence presented by Greenberg, Borsook, Tarver, Zamecnik, and others indicates that incorporation by tissues in the flask probably reflects protein synthesis. Indeed Peters and Anfinsen have obtained from chicken liver slices a radioactive protein which behaved immunologically and electrophoretically like serum albumin (19).

Comparison of these data with other results in animals is difficult because of the different experimental conditions. In our laboratory rat liver slices yielded protein with a specific activity of 250, using the complete Krebs-Henseleit buffer. This is certainly the same order of magnitude as the results with human liver, which was somewhat surprising in view of the report by Sprinson and Rittenberg that the turnover of tissue protein (as

measured by feeding N^{15} labeled glycine) in the rat is five times that of man (20). Whereas under the conditions of the present experiments one specific activity is easily compared with another it must be pointed out that such a comparison does not apply to the specific activities reported by other investigators. In any given experiment the specific activity obtained depends upon the following variables: *1*) the specific activity of the amino acid added at the beginning of the experiment; *2*) the number of hours incubated; *3*) the weight of tissue and its protein concentration; *4*) the efficiency of the counting equipment (the flow-gas counter being three to four times more efficient than the Geiger end-window tube); *5*) the concentration of various ions in the media; and *6*) the concentration of O_2 available. It might nevertheless be of interest to mention several comparative results. Winnick, Friedberg and Greenberg found a comparable depression in uptake by insoluble particles of rat liver homogenate when calcium was omitted from the buffer, but when magnesium was omitted, the degree of inhibition was considerably less than in the current experiments (21). Borsook and his associates reported comparable data (22). Depression of the uptake by a nitrogen atmosphere is the universal finding with all animal tissues with the exception of the uptake of labeled lysine by whole homogenate of guinea pig liver (22). It is likely that nitrogen produced no depression in the experiments using the KCl-$KHCO_3$ buffer because the system was already depressed by operating under sub-optimal conditions.

With the complete buffer the specific activities cluster symmetrically around the mean with a normal type distribution. It is conceivable that the specific activity obtained in C. D. might have been higher had the biopsy been taken earlier in the course of his surgery. The effect of the anaesthesia and the duration thereof are unknown. The marked sensitivity to small amounts of added calcium and magnesium emphasizes the need for carefully defined conditions as a corollary of a "normal" figure.

The incorporation of the labeled glycine into the protein has all the characteristics of an enzymatic reaction which may well be the process whereby the whole organism synthesizes protein. It is obviously an oxygen requiring reaction since it is almost entirely abolished by a nitrogen environment or cyanide. The requirements for added calcium and magnesium ions are striking, and one might infer that phosphorylation is a fundamental part of this mechanism of synthesis. Such data have been reported with experimental animal tissue, and the reports support the conclusion that the requirements include oxygen, adenosinetriphosphate (ATP), magnesium and calcium ions. ATP has been added to animal liver homogenates without significantly increasing the uptake of labeled amino acid. However, Winnick has demonstrated that particles of rat liver will not incorporate labeled glycine after dialysis unless ATP is added (23). Since some glycine is incorporated into protein with the KCl-$KHCO_3$ buffer, it is inferred that the concentration in the human liver slices of some of the components is too low for optimal incorporation, and that this deficiency is easily overcome by adding the desirable components.

Experimental evidence to date is quite convincing that the liver synthesizes serum albumin from the constituent amino acids. This well accepted concept has been reinforced by the recent evidence of Tarver and Reinhardt that the hepatectomized dog incorporates practically no radioactive methionine into serum albumin (24). Low serum albumin concentration is well correlated with advanced hepatic damage and a logical inference is that the liver is incapable of synthesizing albumin at a normal rate. The ability of the liver slice to incorporate radioactive glycine into its protein is quite probably a reflection of the rate of protein synthesis. With this in mind one might expect to find reduced incorporation by pathological liver slices especially where serum albumin concentration is below normal. Such experiments are currently in progress.

SUMMARY

The incorporation of carboxyl C^{14} labeled glycine into the protein of human liver slices has been studied. The specific activities of the protein were higher when the tissue was incubated with Krebs-Henseleit buffer as compared to a KCl-$KHCO_3$ buffer.

The reaction was inhibited by nitrogen, cyanide, and failure to add calcium or magnesium.

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THE DISTRIBUTION OF SODIUM AND POTASSIUM IN MAN [1]

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Simultaneous measurements of body water distribution and total body electrolyte by the isotope dilution technique permit the calculation of total intracellular electrolyte and average intracellular electrolyte concentration. The application of this principle to the measurement of intracellular sodium and potassium in the dog has been reported by Levitt and Gaudino (1). The purpose of this study is to provide comparable data on man.

METHODS

Total body water was determined by the volume of distribution of antipyrine,[2] corrected for metabolism, by the method of Soberman, Brodie, Levy, Axelrod, Hollander and Steele (2). In view of indirect evidence which suggests that antipyrine may not reach maximal distribution in two hours (3), we have preferred, for extrapolation to zero time in correction for metabolism, to use the plasma concentrations from three to seven or eight hours after injection.

Extracellular fluid was measured by the maximal volume of distribution of sucrose as determined by the calibrated infusion technique (4). Intracellular water was calculated as the difference between the antipyrine and sucrose volumes of distribution. Cellular mass was calculated as the difference between body weight and the weight of the sucrose volume of distribution, and total body solid was calculated as the difference between body weight and the weight of the antipyrine volume of distribution.

Total body sodium was determined by the dilution of Na^{24}. The radioactive sodium was administered by a single injection from a calibrated syringe in an amount equal to 1.3 μc. per kg. of body weight. Radiation incident to the administration of this amount of Na^{24} does not exceed the accepted tolerance dose of 0.1 r/day (5). A period of 24 hours was allowed for complete distribution of the sodium isotope and during this interval all urine was collected. The plasma specific activity (counts/min. per mEq. plasma sodium) at 24 and 25 hours was calculated from the counts/min. per cc. of plasma and the concentration of sodium in plasma (in mEq./cc.) as determined against an internal lithium standard with a Perkin-Elmer flame photometer. After application of the appropriate corrections for radioactive decay, total body sodium (in mEq.) was calculated from the difference between the Na^{24} injected and excreted, divided by the equilibrium specific activity of the Na^{24}. Extracellular sodium was calculated as the product of plasma sodium concentration (mEq./liter) and extracellular fluid volume (liters).[3] Total intracellular sodium was calculated as the difference between total body sodium and extracellular sodium. Intracellular sodium is expressed as concentration per unit of intracellular water (mEq./liter), per unit of cellular mass (mEq./kg.) or per unit of body solid (mEq./kg.).

Total body potassium was determined by the dilution of K^{42}. The radioactive potassium was administered by a single injection from a calibrated syringe in an amount equal to 1.9 μc. per kg. of body weight, a dose which does not produce radiation in excess of the accepted tolerance dose of 0.1 r/day (5). A satisfactory method for preparing the radioactive potassium for injection has been described by Corsa, Olney, Steenburg, Ball and Moore (6). In accordance with the data of these investigators a period of 40 hours was allowed for maximum distribution of the radioactive potassium and during this interval all urine was collected. The specific activity of the plasma was taken as equal to that of the urine (in counts/min. per mEq. of potassium) which was determined on urine samples collected at 36, 40 and 44 hours. Total body potassium was calculated from the difference between the K^{42} injected and excreted, divided by the equilibrium urine specific activity of the K^{42}. Extracellular potassium, intracellular potassium and intracellular potassium per unit of cell water, cell mass and body solid were calculated in a manner similar to that described for sodium.

[1] This study was supported by grants from the Atomic Energy Commission (AEC Contract AT [30-1]-1025) and the Commonwealth Fund.

We are indebted to the Fourth (New York University Post-Graduate Medical School) Medical and Surgical Divisions of Bellevue Hospital for the privilege of studying patients on these services.

[2] The antipyrine used in these studies was supplied in ampoule form through the courtesy of Eli Lilly and Company, Indianapolis 6, Indiana.

[3] A more accurate method of calculation would take into account the volumes of the plasma (PV) and extracellular fluid (ECF), the water content of the plasma (w) and the Donnan factor, k. Taking PV as 4 per cent and ECF as 16 per cent of the body weight, respectively, w as 0.92 and k as 0.95, the calculation as given in the text yields a value for extracellular sodium which is 1.8 per cent too large. Because of its small order this correction has been neglected here.

TABLE I

Intracellular potassium in six subjects

Patient	Age	Sex	Height	Weight	Diagnosis	Total body water	Extracellular fluid	Intracellular water	Total body solid	Cellular mass	Plasma potassium	Total potassium	Extracellular potassium	Intracellular potassium	Intracellular potassium concentration		
															Per liter cell water	Per kg. body solid	Per kg. cell mass
	yrs.		*in.*	*kg.*		*liters*	*liters*	*liters*	*kg.*	*kg.*	*mEq./l.*	*mEq.*	*mEq.*	*mEq.*	*mEq./l.*	*mEq./kg.*	*mEq./kg.*
K. O.	43	M	67	70.5	Healed burn	36.4	12.4	24.0	34.1	58.1	4.3	2,900	50	2,850	119	84	56
J. M.	44	M	65	72.7	Ear deformity	33.6	12.8	20.8	39.1	59.9	4.2	2,660	60	2,600	125	66	43
W. J.	30	M	63	64.1	Inguinal hernia	38.4	12.5	25.9	25.7	51.6	4.8	2,880	60	2,820	109	110	55
L. I.	15	M	62	47.7	Subcutaneous lipoma	29.7	8.7	21.0	18.0	39.0	4.0	2,050	30	2,020	96	112	52
C. S.	18	F	60	49.5	Subcutaneous fibroma	23.9	9.8	14.1	25.6	39.7	4.4	1,630	40	1,590	113	62	40
F. G.	25	M	65	66.8	Non-toxic thyroid adenoma	35.5	9.6	25.9	31.3	57.2	4.5	2,870	40	2,830	109	90	49
Average															112	87	49

All radioactivity measurements were performed in duplicate on 1 cc. samples which were dried in aluminum planchettes and counted with a shielded end-window Geiger tube. For uniform statistical accuracy, all samples were counted to a 95 hundredths error (7) of less than 5 per cent. Usually, the 95 hundredths error was less than 3 per cent.

The subjects were patients, ranging in age from 13 to 58 years, who were hospitalized for elective surgery except J. T., who was studied 10 days after appendectomy.

RESULTS AND DISCUSSION

Table I presents the simultaneous measurements of body water compartments and total body potassium in six subjects (five male and one female). The calculated values for total intracellular potassium and the concentration of potassium in intracellular water are shown for each subject. Intracellular potassium averaged 112 mEq./liter (range 96 to 125). This value is in good agreement with the average intracellular potassium concentration from four studies in the dog which was 115 mEq./liter (range 109 to 123) (1). No other data on intracellular potassium concentration in man are available. However, Corsa and his coworkers (6) have estimated the intracellular potassium concentration to be 106 mEq./liter (by calculation from an assumed value for the volume of extracellular fluid and measurements of total body water and total body potassium in man).

From the data presented in Table I, the values for intracellular potassium per unit of cellular mass and per unit of body solid may be calculated. Intracellular potassium averaged 49 mEq./kg. (range 40 to 56) of cellular mass and 87 mEq./kg. (range 62 to 112) of body solids.

TABLE II

Intracellular sodium in six male subjects

Patient	Sex	Age	Height	Weight	Diagnosis	Total body water	Extracellular fluid	Intracellular water	Total body solid	Cellular mass	Plasma sodium	Total sodium	Extracellular sodium	Intracellular sodium	Intracellular sodium concentration		
															Per liter cell water	Per kg. body solid	Per kg. cell mass
		yrs.	*in.*	*kg.*		*liters*	*liters*	*liters*	*kg.*	*kg.*	*mEq./l.*	*mEq.*	*mEq.*	*mEq.*	*mEq./l.*	*mEq./kg.*	*mEq./kg.*
A. S.	M	58	63	64.6	Inguinal hernia	32.0	10.7	21.3	32.6	53.9	132	2,200	1,410	790	37.1	24.2	14.7
A. M.	M	41	72	84.5	Inguinal hernia	43.7	14.4	29.3	40.8	70.1	137	3,130	1,970	1,160	39.6	28.4	16.5
K. L.	M	49	65	51.3	Hemorrhoids	36.1	11.1	25.0	15.2	40.2	136	2,600	1,510	1,090	43.5	71.7	27.1
C. F.	M	22	71	73.6	Anal fissure	44.8	14.1	30.7	28.8	49.5	138	2,910	1,950	960	31.3	33.3	16.1
J. D.	M	56	64	61.8	Peptic ulcer	34.6	15.5	19.1	27.2	46.3	130	2,660	2,020	640	33.5	23.5	13.8
J. T.	M	13	54	37.2	Post-appendectomy (10 days)	20.3	5.2	15.1	16.9	32.0	136	1,260	705	555	36.8	32.8	17.3
Average															37.0	35.7	17.6

Table II contains the data from the simultaneous measurements of body water compartments and total body sodium in six male subjects. For each subject, the calculated values for total intracellular sodium and concentration of sodium in intracellular water are indicated. The average intracellular sodium concentration for these subjects is 37.0 mEq./liter (range 31.3 to 43.5), a figure close to the average of 36.0 mEq./liter (range 27.5 to 41.0) obtained from four studies in the dog (1). No comparable data are available in man; however, Forbes and Perley (8) have measured total body sodium by dilution of Na^{24} in 32 males and obtained an average of 41.9 mEq./kg. of body weight, a figure to be compared with our average of 37.9.

Values have been derived for intracellular sodium per unit of body solid from the information presented in Table II. Intracellular sodium averaged 17.6 mEq./kg. (range 13.8 to 27.1) of cellular mass and 35.7 mEq./kg. (range 23.5 to 71.7) of body solid.

A direct investigation of the error of measurement by the isotope dilution technique involves the use of total desiccation procedures. Therefore the accuracy of these values for intracellular sodium and potassium in man can only be evaluated in an indirect manner by estimation of the normal values in man and by a comparison which has been made by Corsa and his co-workers (6). They have presented studies of tissue specific activity in rabbits and man which indicate that the total potassium as measured after a 40 hour period of equilibration is within 5 per cent of the actual amount of total body potassium. However, the precise time required for equilibrium distribution of radioactive sodium is not known. It is probable that a certain portion of body sodium (*i.e.*, bone sodium) has not equilibrated in 24 hours, the interval employed in this study.

It should be emphasized that the electrolyte concentrations obtained by these calculations are average concentrations representing all the tissues of the body, and not the concentration in any single tissue.

SUMMARY

Intracellular sodium and potassium have been calculated from simultaneous studies of body water distribution (total body water and extracellular fluid) and total body electrolyte as measured by the isotope dilution technique. The concentration of intracellular electrolyte has been calculated per unit of intracellular water, per unit of cellular mass and per unit of body solid.

Intracellular potassium averaged 112 mEq./liter (range 96 to 125) of intracellular water, 49 mEq./kg. (range 40 to 56) of cellular mass, and 87 mEq./kg. (range 62 to 112) of body solid.

Intracellular sodium averaged 37.0 mEq./liter (range 31.3 to 43.5) of intracellular water, 17.6 mEq./kg. (range 13.8 to 27.1) of cellular mass, and 35.7 mEq./kg. (range 23.5 to 71.7) of body solid.

The average values for intracellular sodium and potassium concentration in man, as determined in this study, are in close agreement with comparable data obtained from studies in the dog and with estimates of intracellular electrolyte in man.

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THE DISTRIBUTION OF TOTAL BODY CHLORIDE IN MAN [1]

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The studies of Weir (1), Weir and Hastings (2), and Wallace and Brodie (3) have demonstrated the similarity of bromide and chloride distribution in tissues and body fluids of dogs and cats. With the exception of the brain and cerebrospinal fluid (4), bromide enters tissue in proportion to the amount of chloride present and after equilibration the ratio of the concentrations of tissue bromide to chloride is the same as in the plasma.[2] In effect, administered bromide is diluted in body chloride and not in any specific compartment of body water. Thus, the extent of bromide dilution can be used at least as an approximate measure of total body chloride. Measurements of total body chloride, using bromide dilution, in animals and man have been reported by Weir (5) and Dunning, Steele and Berger (6). No data are available, however, on intracellular chloride concentration since previous studies have not included simultaneous measurement of extracellular and intracellular water. The following studies supply data of this type.

METHODS

Six males and one female without evidence of edema were used in this study. Total body water and extracellular fluid volume were determined with sucrose and antipyrine,[3] respectively, as described in a preceding paper (7). Intracellular water was calculated as the difference between the antipyrine and sucrose volumes of distribution. Total body solid was calculated as the difference between body weight and the weight of the antipyrine volume of distribution, and cellular mass as the difference between body weight and the weight of the sucrose volume of distribution. Bromide was determined by the method of Brodie and Friedman (8) and chloride by the method of Wilson and Ball (9). The bromide method was checked by analysis of plasma solutions of known concentration and gave an average recovery of 99 (range 98 to 102) per cent.

Sodium bromide was administered by a single injection from a calibrated syringe or burette in an amount equivalent to 1.5 to 2.5 gm. of bromide. A period of 24 hours was allowed for complete equilibration of bromide before the final blood sample was obtained. Urinary bromide excretion was determined throughout this equilibration period, and utilized to calculate the bromide retained in the body.[4]

Total body chloride, as determined by bromide dilution was calculated as:

$$\text{Total body chloride} = \text{plasma chloride concentration} \times \frac{\text{retained bromide}}{\text{plasma bromide concentration}}.$$

In calculating the extracellular chloride (ECF_{Cl}) account must be taken of the plasma volume (PV), Donnan factor (k), plasma water content (w), and extracellular fluid volume (ECF), in accordance with the following equation:

$$f = \frac{PV + \frac{k}{w}[ECF - PV]}{ECF_{Cl}\,P_{Cl}}.$$

We have not measured PV, k and w, but we may with reasonable accuracy take PV as 4 per cent of the body weight, k as 1.02 and w as 0.92. Inserting these values and the observed values of ECF yields the individual factors listed under f in Table I, which have been used in the calculation of ECF_{Cl}.

RESULTS AND DISCUSSION

Table I contains the data from simultaneous measurements of body water compartments and total body chloride in seven subjects. Total chloride ranges from 27.1 to 33.7 and averages 30.1 mEq./kg. body weight, figures which may be com-

[1] This study was supported by grants from the Atomic Energy Commission (AEC Contract AT [30-1]-1025) and The Commonwealth Fund to the Department of Physiology and by a grant from the Masonic Foundation for Medical Research to the Study Group for Rheumatic Diseases.

We are indebted to the Fourth (New York University Post-Graduate Medical School) Medical and Surgical Divisions of Bellevue Hospital for the privilege of studying patients on these Services.

[2] However, in red blood cells the ratio bromide/chloride may be slightly greater than plasma (2).

[3] The antipyrine was supplied in ampoule form through the courtesy of Eli Lilly & Company, Indianapolis, Indiana.

[4] The plasma chloride determination was not corrected for the presence of bromide since the equilibrium bromide concentration was usually less than 2 per cent of total plasma halide.

TABLE I

Intracellular chloride in seven subjects

Patient	Age	Sex	Height	Weight	Diagnosis	Total body water	Extracellular fluid	Intracellular water	Total body solid	Cellular mass	Plasma chloride	Total chloride	Total chloride	f	Extracellular chloride	Intracellular chloride	Intracellular / Total chloride	Intracellular chloride concentration		
																		Per liter cell water	Per kg. body solid	Per kg. cell mass
	yrs.		*in.*	*kg.*		*liters*	*liters*	*liters*	*kg.*	*kg.*	*mEq./l.*	*mEq.*	*mEq./kg.*		*mEq.*	*mEq.*		*mEq./l.*	*mEq./kg.*	*mEq./kg.*
A. M.	41	M	72	84.5	Inguinal hernia	43.7	14.4	29.3	40.8	70.1	113	2,630	31.1	1.085	1,766	865	.329	29.5	21.2	12.3
C. E.	22	M	71	73.6	Anal fissure	44.8	14.1	30.7	28.8	59.5	114	2,480	33.7	1.094	1,759	722	.291	23.5	25.1	12.1
A. S.	58	M	63	64.6	Inguinal hernia	32.0	10.7	21.3	32.6	53.9	112	1,770	27.4	1.082	1,297	473	.267	22.2	14.5	8.8
J. M.	44	M	65	72.7	Ear deformity	33.6	12.8	20.8	39.1	59.9	108	2,060	28.3	1.087	1,503	557	.271	26.8	14.3	9.3
L. I.	15	M	62	47.7	Subcutaneous lipoma	29.7	8.7	21.0	18.0	39.0	108	1,510	31.7	1.089	1,023	487	.322	21.2	27.0	16.2
C. S.	18	F	58	49.5	Subcutaneous fibroma	23.9	9.8	14.1	25.6	39.7	115	1,560	31.5	1.110	1,251	309	.198	21.9	12.1	7.8
F. G.	25	M	65	66.8	Non-toxic thyroid adenoma	35.5	9.6	25.9	31.3	57.2	104	1,810	27.1	1.080	1,078	732	.404	28.3	23.4	12.8
Average																		24.8	19.7	11.3

pared with a range of 27.9 to 35.2 and an average of 31.6 in five males without edema reported by Dunning, Steele, and Berger (6). These investigators obtained the slightly lower value of 28.6 mEq./kg. in five females. Total body chloride in cats, dogs and rabbits, as measured by bromide dilution, averaged 34.4, 33.2 and 30.2 mEq./kg., respectively (5), values which are in close agreement with measurements of total body chloride as determined from analyses of homogenized aliquots of animals (10).

Intracellular chloride averaged 24.8 mEq./liter of cell water (range 21.2 to 29.5). This figure is, of course, a nominal one and does not reveal the true chloride concentration in any one tissue. No data are available for comparison with this value, nor can its accuracy be checked directly.

Intracellular chloride averaged 11.3 mEq./kg. of cell mass (range 7.8 to 16.2) and 19.7 mEq./kg. of body solid (range 12.1 to 27.0).

Dunning, Steele and Berger (6) surmise that about half the body chloride is in the cells, but our data show that the intracellular fraction ranges from 19.8 to 40.4 per cent of the total chloride, with an average ratio of 29.7. However, we concur with their statement that a change in total chloride does not necessarily represent a change in volume of extracellular fluid. The older assumption that chloride is confined to the extracellular fluid must, we think, be abandoned in the light of the present studies, and in view of the excessively large chloride spaces reported by Dunning, Steele and Berger in man (6), by Burch, Threefoot, and Ray (11) in the dog, the high bromide space/inulin space ratio (1.30 to 2.40) reported by Berger, Dunning, Steele, Jackenthal and Brodie (12), and the direct analyses of Manery and Hastings (13), Crismon, Crismon, Calabresi and Darrow (14) and Wilde (15). The evidence indicates that intracellular chloride is highly variable in amount, and one may infer from this variability and the viviperfusion experiments of Amberson, Nash, Mulder and Binns (16) that it is also highly labile. Calculation of changes in the extracellular fluid volume from changes in the total body chloride must therefore have an uncertain value.

SUMMARY

Intracellular chloride has been determined from simultaneous studies of body water distribution (total body water and extracellular fluid) and total body chloride as measured by the bromide dilution method in seven non-edematous subjects.

Total chloride averaged 30.1 mEq./kg. body weight (range 27.1 to 33.7). Intracellular chloride averaged 29.7 per cent of total chloride (range 19.8 to 40.4), and 24.8 mEq./liter of cell water (range 21.2 to 29.5), 11.3 mEq./kg. of cell mass (range 7.8 to 16.2), and 19.7 mEq./kg. of body solid (range 12.1 to 27.0).

In view of the large fraction of total body chloride which is contained in the cells, changes in total body chloride cannot be accepted as necessarily reflecting changes in extracellular fluid volume.

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OXYGEN TENSION OF TISSUES BY THE POLAROGRAPHIC METHOD. IV. SKIN OXYGEN TENSION VS. ARTERIAL OXYGEN SATURATION; RELATIONSHIP TO OXYHEMOGLOBIN DISSOCIATION CURVE

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INTRODUCTION

The relationship between the oxygen saturation and oxygen tension of the blood has, up to the present time, been studied by methods requiring *in vitro* analysis. These methods, such as the Van Slyke-Neill manometric (1) and Krogh equilibration (2) techniques, require considerable time and skill and give only discontinuous measurements. This report describes an indirect study of this relationship by simple instrumental methods which supply immediate and continuous information *in vivo*. They consist of the oxygen electrode (3), which can be used to measure polarographically the oxygen tension in the intact human skin (4), and the oximeter (5, 6), which measures the arterial blood oxygen saturation in the intact human ear.

In this paper are described the changes in the oxygen tension of skin warmed by a rapid circulation, brought on by altering the blood arterial oxygen saturation in normal human subjects (7). The correlation of skin oxygen tension and blood arterial saturation and its relationship to the oxyhemoglobin dissociation curve are demonstrated.

METHOD

The method of producing alterations of the blood arterial oxygen saturation has been described previously (7). It consists of administering constantly varying mixtures of pure oxygen and pure nitrogen through an anesthesia machine to obtain and maintain the desired level of the arterial oxygen saturation, as measured by the Millikan oximeter.

The subject, shortly after eating his regular meal, lay quietly for approximately 45 minutes, breathing air, before inhaling the gas mixtures. Skin temperature readings were taken, the oximeter was attached to the ear, and the oxygen electrodes were inserted in the skin. "Ear-thickness" readings were taken on the oximeter until stable and the "saturation" was then set at "100" while the subject breathed pure oxygen (5, 6). Oxygen tension readings were taken at frequent intervals until they became stable. A low or high oxygen mixture was then inhaled. After the inhalation of this gas mixture, the subject was again allowed to breathe room air. In some experiments the arterial saturation and the oxygen tension changes during this period of recovery were noted.

The saturations studied ranged from 67 to 100%. Any change from one level of saturation to another was made gradually, and the saturation was kept constant at the desired level for several minutes. While the saturation was held constant, oxygen tension readings were taken, in most experiments until they too became constant (solid circles, Figure 1). In some of the experiments, where the effect of reduced arterial oxygen saturation was being studied, repeated oxygen tension readings were not taken (open circles, Figure 1) at each level of saturation in order not to prolong unduly the total period of hypoxia. In these instances readings were taken only after the desired saturation had been maintained for a period of time found, from the other experiments, to be sufficient for the skin oxygen to come into equilibrium with the blood arterial oxygen.

The oxygen electrode, method of taking readings, and corrections for temperature variations have been described by Montgomery and Horwitz (4). In the present experiments, however, the electrodes were uncalibrated so that only relative changes were measured. The reading obtained while the subject was breathing air was arbitrarily designated at 100. The electrodes were sterilized by boiling and their tips were kept in sterile saline solution when not in use. The electrodes were inserted in the skin of the outer aspect of the arm of each subject. Eight different electrodes were used and, in most of the experiments, the same set of five electrodes.

The first three experiments, in which only two electrodes were used, showed that the oxygen tension percentages of the two electrodes, at a given arterial saturation, differed significantly. To minimize the effect of these differences, from three to five electrodes were used in all subsequent experiments, the oxygen tension (Figure 1) being taken as the average of values obtained by the several electrodes.

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Skin temperature was recorded continuously throughout each experiment by thermocouples (8) placed on different areas of the skin near the various electrodes. In order to maintain a rapid circulation through the skin of the arm it was occasionally necessary to apply heat elsewhere to the body. The skin temperatures were kept between 30 and 34° C except in two instances where the temperature of the skin could not be raised above 29° C. The room temperature of all experiments averaged 26° C. Neither skin nor room temperature varied more than one degree throughout any one experiment.

Fourteen normal, white adults, ages 18 to 46, were used as subjects; all but three were males. A total of 31 experiments was performed.

OBSERVATIONS

The effect of decreasing or increasing the blood arterial oxygen saturation on the oxygen tension of the skin is shown in Figure 1, where the results of the 31 experiments are plotted. In each experiment the oxygen tension changed in the same direction as the arterial saturation.

One can see in Figure 1 striking differences in the amount of change in oxygen tension, per unit change in arterial saturation, at different levels of arterial saturation. In lowering the saturation from 75 to 70%, for instance, the tension was decreased by only 4%. In lowering the saturation from 95 to 90%, the tension, on the other hand, was decreased by 20%. The large changes in tension at the high arterial saturations were especially apparent in several instances (not included in Figure 1) where the arterial saturation was progressively increased between 96 and 99% saturation by small increments ranging from ¼ to 1% arterial saturation. These small changes in saturation, regardless of how gradually they were made, always produced considerable increases in oxygen tension.

Several other observations on the relationship between the arterial oxygen saturation and skin oxygen tension were made:

1. The recovery of the oxygen tension and the arterial oxygen saturation: Observations on the rate of recovery of the arterial saturation and the skin oxygen tension were made when the subject was allowed to breathe room air immediately upon the termination of some of the experiments. After low oxygen inhalation (two experiments), the arterial saturation fully regained its normal value in approximately five minutes while the skin oxygen tension required about 10 minutes. After high oxygen (six experiments), the saturation also returned to normal in approximately five minutes and the tension again required twice as much time.

The final recovery value of the arterial saturation, noted in most instances after the subject had

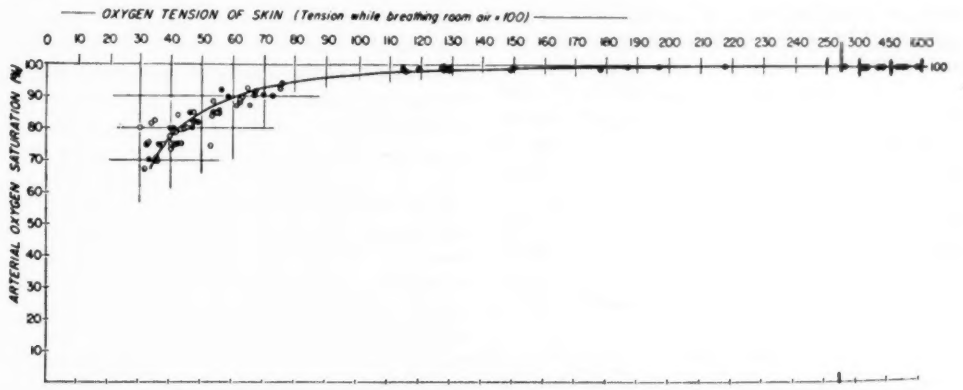

FIG. 1. SKIN OXYGEN TENSION VERSUS ARTERIAL OXYGEN SATURATION

The skin oxygen tension of each of the points was obtained by averaging the values of several electrodes used simultaneously.

● Skin oxygen tension in equilibrium with arterial oxygen as obtained by repeat tension readings at each level of arterial saturation (see Method).

○ Skin oxygen tension in equilibrium with arterial oxygen as obtained by single tension readings at each level of arterial saturation after the saturation had been maintained constant for several minutes (see Method).

TABLE I

Oxygen tension of the skin, in galvanometer units, while subject is breathing air

Values "For each experiment" are average of all electrodes used in each experiment. One very high reading, 88, obtained with electrode "B," was not included. Readings were obtained after electrodes had been in the skin for approximately 30 minutes and were stable.

	For each experiment	For each electrode				
		A	B	C	D	E
Oxygen tension Average	20	19.2	25.3	18.2	17.2	16.8
Stand dev.	4.6	3.2	9.4	6.1	8.7	5.6
Range	12 to 27	15.5 to 29	12.5 to 38	7.5 to 29	8.5 to 32.5	6.5 to 26
No. experiments	13	12	12	12	12	8

been breathing room air for approximately 15 minutes, was about the same as the initial reading on room air. The oxygen tension on recovery from breathing low oxygen averaged 91% (range 73 to 108%) of the initial room air reading, whereas it was 106% (range 86 to 138%) after the inhalation of pure oxygen.

In a few instances a droplet of blood was found when the electrodes were removed. This had no apparent effect on the readings, unlike the experience of Montgomery and Horwitz (4).

2. Effect of carbon dioxide: In a single experiment, in which the arterial saturation was maintained at 80%, 2.5% CO_2 (9), included in the gas mixture for a period of 10 minutes, caused no measurable change in the oxygen tension.

3. Skin oxygen tension in galvanometer units while breathing air: It was noted throughout these experiments that the oxygen tension reading of skin, warmed by a rapid circulation, was fairly consistent while the subjects were breathing air. The readings for the five electrodes used most frequently were analyzed and are listed in Table I. The average value of each of these five electrodes ranged between 16.8 and 25.3 galvanometer units.

DISCUSSION

Under the conditions of these experiments, in which an attempt was made to maintain a rapid cutaneous circulation, the oxygen tension of the skin, measured polarographically by the oxygen tension electrode, changed consistently with the blood arterial oxygen saturation, as measured by the Millikan oximeter.

The quantitative relationship between the tension, expressed in percent of initial value while breathing air, and the saturation may be represented by a curve (Figure 1) which, between 67% arterial saturation and the upper limit of the oxyhemoglobin dissociation curves obtained by gasometric analysis (10–12), has the characteristically changing slope of these latter curves. At the higher saturations in the present experiments, the relationship is closely similar to that reported by direct polarographic measurements on the arterial blood (13). The oxygen tension of the skin having a fast circulation thus bears the same type of relationship to the arterial oxygen saturation as does the arterial oxygen tension.

Some technical and physiological limitations of these experiments may be emphasized: *1.* The observations extend down to only 67% arterial saturation. Hypoxemic patients may offer an opportunity to study the curve at lower levels. *2.* Certain limitations are inherent in the Millikan oximeter (5, 6, 14) and in the stationary platinum oxygen electrode (4). When the arterial oxygen saturation was increased, or decreased, to any given level, there was a considerable difference between tension changes of individual electrodes. These differences are unexplained and demonstrate the importance of using several electrodes in determining the degree of any change. *3.* An attempt was made to maintain a cutaneous circulation fast enough (as indicated by differences between skin temperature and room temperature) to supply oxygen in excess of metabolic needs. Variations in cutaneous circulation or oxygen metabolism are expected to contribute some error (4). *4.* Two important physiological factors, the pH (6) and the temperature (15, 16) of the blood and tissues, were not completely controlled or measured. It is of interest to note that the scatter of observations in Figure 1 closely resembles the scatter of points of the conventional dissociation curve having alterations of the blood pH over the range 7.2 to 7.6 (6). Considering the above, the curve may prove to be more useful if some of these variables are better controlled.

By the simultaneous use of the oximeter and oxygen electrode, clinical disturbances related to the oxygen dissociation of the blood may be more readily laid open to investigation. The effect of various metabolic and hypoxemic states on the

oxyhemoglobin dissociation of blood may be studied at the bedside of critically ill patients without discomfort or the loss of blood.

Two other observations on the relationship between the arterial oxygen saturation and the oxygen tension of the skin were made: *1.* The oxygen tension of the skin, after the inhalation of low or high oxygen mixtures, required several minutes longer than the arterial saturation to return fully to its original value. This agrees with other studies made on the viscera of animals (17). *2.* The consistency of all the oxygen tension galvanometric readings on our subjects, while they were breathing air (Table I), suggests that the uncalibrated oxygen electrode may be used to establish an "oxygen tension reading," in galvanometric units, of skin with a rapid circulation in normal subjects. Such an electrode may then be useful in studying changes in oxygen tension in skin and their implications regarding the central and peripheral circulation.

SUMMARY

1. The blood arterial oxygen saturation of normal human subjects was altered by the inhalation of a gas of varying oxygen concentration to give readings between 67 and 100%, as measured by the Millikan oximeter. Simultaneously the oxygen tension of the skin, having a reasonably rapid circulation, was measured by several oxygen electrodes.

2. The oxygen tension changed in the same direction as did the arterial saturation. When the tension, expressed in percent of the reading obtained while breathing air, was plotted against the arterial oxygen saturation, a curve was obtained (Figure 1) with the characteristically changing slope of the conventional oxyhemoglobin dissociation curves of blood.

3. After the inhalation of low or high oxygen mixtures, the skin oxygen tension returned more slowly to initial values than did the arterial oxygen saturation.

4. Unexplained differences in the tension changes between the individual electrodes made the simultaneous use of several electrodes advisable.

5. Oxygen tension galvanometric readings obtained while breathing air were fairly consistent, suggesting that the uncalibrated oxygen electrode may be used to establish an "oxygen tension reading" of skin with a rapid circulation in normal subjects.

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A COMPARISON OF THE ESTIMATION OF THE BASAL CARDIAC OUTPUT FROM A LINEAR FORMULA AND THE "CARDIAC INDEX" [1]

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It is customary to express the cardiac output of man in units of body size, usually in the form of the cardiac index which is simply the gross cardiac output in liters of blood per minute divided by the surface area of the body. The use of the cardiac index implicitly assumes that the cardiac output normally is directly proportional to the surface area of the body with the prediction line going through the origin of the X and Y axes. Tanner (1, 2) has recently pointed out that errors arise in using cardiac index as a criterion for prediction of cardiac output when strict proportionality is not present. In this situation, the regression line which describes the best linear fit for any group of data differs from the line defined by the cardiac index. Under these conditions the cardiac index will overestimate or underestimate the expected value for an individual in the extreme range of the body size and comparisons of population groups with reference to the cardiac index will be inexact unless the surface areas of the two population groups are identical. Tanner (1) used the relationship of stroke volume to body weight as an example of such errors but did not provide an analysis of the errors involved in the cardiac index.

A large percentage of the cardiac output data in the literature is expressed in terms of the cardiac index. Recently standards for the low-frequency, critically damped ballistocardiograph have been published exclusively in terms of this ratio by Gregersen and Nickerson (3). It is the purpose of this paper to examine the validity of the cardiac index as a standard with special reference to its usefulness in predicting the cardiac output of individuals.

SUBJECTS AND CONDITIONS

Cardiac output and surface area data were obtained on 48 healthy, male, university students who were between the ages of 18 to 30. All of these men were studied with the low-frequency, critically damped ballistocardiograph. Duplicate records were made in each case. Ten cardiac complexes were analyzed in each record. The cardiac output of 34 of these men was determined with the acetylene method. Quadruplicate determinations by this method were made in 22 men, duplicates in 12. All men reported to the Laboratory without breakfast and lay on a bed for 20 minutes before observations were begun. All observations were carried out in an air conditioned room which was maintained at a temperature of $78 \pm 2°$ F. The subjects were studied during the months of October, November, December, March and April.

METHODS

Cardiac output estimations by the acetylene method were made following the Grollman procedure. The final computation applied the correction given by Chapman and associates (4) so that the absolute values are in harmony with those obtained from the direct Fick (catheter) method.

Cardiac output estimations by the ballistocardiographic method employed the low-frequency, critically damped apparatus described by Nickerson and Curtis (5). The computational method was that of Nickerson, Warren and Brannon (6); this has also been calibrated against the catheter method. The surface area of all subjects was determined by the formula of Dubois.

SELECTION OF DATA FROM THE LITERATURE

Data in the literature which may be used for the purpose of this discussion have been reviewed by Tanner (1). In the present analysis we have included the two series presented by Tanner in which the cardiac output was determined by the high-frequency ballistocardiograph on males, the acetylene method and the ethyl iodide method. Tanner pointed out, from statistical considerations, that the two series of measurements on normal individuals which were carried out by the direct Fick method appear to have been drawn from different populations. One of these series of measurements, which was collected by Cournand's group (7), was carried out in New York and the other by Stead, Warren, Merrill, and Brannon (8) was done in Atlanta, Georgia. Since the publication of Tanner's work, data on 19 normal males age 20 to 40 have been gathered in the same laboratory in Minneapolis by Chapman and associates (4) and Ebert, Borden, Wells, and Wilson (9). It seems reason-

[1] This work was supported in part by the Research Grants Division of the U. S. Public Health Service.

able to assume that at least part of the difference between the data obtained in Atlanta and New York was due to climate. We have, therefore, excluded the Atlanta data and pooled the Minneapolis data and that part of the New York data obtained on young men between the ages of 20 to 40.

STATISTICAL METHODS

Methods for calculating standard deviations, correlation coefficients, and constants for regression functions can be found in any elementary textbook of statistics. A few methods have been employed which should be stated in some detail to avoid confusion.

The first of these has to do with testing the significance of correlation coefficients. For this purpose we have used Fisher's z transformation (10). This was done with the following equation:

$$(1) \qquad z = \ln_e \left\{ \frac{1 + r}{1 - r} \right\}^{\frac{1}{2}},$$

where r is the correlation coefficient. When this is done, z is normally distributed with variance equal to $1/(n - 3)$.

TABLE I

Basal cardiac output data obtained with the low-frequency, critically damped ballistocardiograph along with some of the characteristics of the subjects

Subject	Age	Height	Weight	Blood pressure	Heart rate	Stroke volume	Cardiac output
	(*years*)	(*cms.*)	(*kgs.*)				
CVD 104	23	163	53.0	115/82	68	82.5	5.61
CVD 3	25	178	58.5	110/80	69	76.2	5.26
Scan.	19	181.6	54.9	104/60	70	87.7	6.14
Forth.	29	175.9	65.5	118/70	60	109.3	6.56
CVD 13	22	176	70.1	115/72	54	114.4	6.18
CVD 56	24	168	51.7	110/66	65	78.9	5.13
Willem.	20	175.9	60.5	114/78	65	103.5	6.73
Erick.	20	178.1	55.5	110/66	58	97.5	5.66
Traut.	22	179.1	69.5	120/73	67	97.5	6.53
CVD 119	20	178	71.5	172/66	70	110.5	7.74
CVD 17	22	178	69.2	103/75	54	90.7	4.90
Strau.	21	170.8	71.8	125/85	64	129.2	8.27
CVD 162	24	181	73.5	126/82	64	116.9	7.48
CVD 7	25	166	67.0	122/75	54	92.0	4.97
CVD 41	25	168	76.5	111/70	55	92.7	5.10
Svig.	25	178.4	77.2	145/85	70	130.0	9.10
Torger.	24	178.4	76.1	124/76	62	122.9	7.62
Navya	28	165.1	54.1	110/76	76	86.3	6.56
CVD 120	24	175	79	139/84	73	121.6	8.88
Alterm.	20	168.3	73.4	114/73	64	112.0	7.17
CVD 110	20	174	85	118/83	60	102.8	6.17
Ortlip.	20	167	80.7	109/72	50	121.2	6.06
CVD 31	26	174	95.5	119/89	69	92.2	6.36
Erwi.	27	183.5	102.0	120/80	71	154.5	10.97
Podem.	23	173.7	89.5	104/67	59	115.2	6.80
Duckw.	24	183.5	135.2	126/83	74	144.2	10.67
Teitenb.	19	189.2	83.7	106/73	66	125.2	8.26
Boss.	25	182.9	95.9	122/78	66	100.5	6.63
Dav.	25	173	73.0	121/78	62	112.3	6.96
Wies	20	181.6	84.9	144/87	56	139.2	7.80
Christen.	21	179.1	84.0	126/80	56	106.8	5.98
Evang.	17	177.8	66.8	118/72	59	101.5	5.99
Lindl.	21	174.6	62.2	120/77	59	88.1	5.20
Hof.	24	172.1	70.1	125/82	61	121.6	7.42
Carls.	24	178.4	62.3	122/66	72	92.7	6.68
Donald.	20	184.2	71.1	119/70	57	95.8	5.46
Koch.	24	174.6	76.6	120/82	77	86.3	6.64
Adam.	23	171.5	75.5	130/84	65	119.1	7.74
CVD 21	24	176	78.3	126/82	63	82.2	5.18
CVD 23	26	174	77.2	121/83	51	100.4	5.13
CVD 30	22	185	74.5	120/71	61	112.0	6.85
CVD 31	26	174	95.5	119/79	69	92.1	6.36
CVD 45	24	188	85.2	112/75	79	87.2	6.89
CVD 50	22	183.0	79.0	134/91	64	133.2	8.53
CVD 59	22	183.0	71.5	140/88	63	94.7	5.97
CVD 109	24	182.0	93.3	155/88	63	154.1	9.71
CVD 144	21	170	63.5	119/80	65	81.0	5.27
CVD 145	19	176	96	132/86	76	87.0	6.61
Mean	22.8	176.4	75.7	122.0/77.5	64.1	106.2	6.79
S. D.	2.59	5.99	15.1	13.9/ 7.2	6.98	19.7	1.43

The root mean square of sums of deviations of observations from corresponding hypothetical values is given by

$$(2) \qquad \left\{\frac{\Sigma(Y - \tilde{Y})^2}{n}\right\}^{\frac{1}{2}},$$

where Y is the observed value and $\tilde{Y}$ is the corresponding hypothetical value.

When Y equals the mean of the observed values ($\overline{Y}$) expression (2) reduces to the sample estimate of the standard deviation, s_Y.

The cardiac index line is defined by the equation

$$(3) \qquad \hat{Y} = kX,$$

where $k = \overline{Y}/\overline{X}$. Then the root mean square of deviations from this line is given by the special case of expression (2), where $\tilde{Y} = \hat{Y}$. After some manipulation this expression reduces to

$$(4) \qquad [s_Y^2 - 2 \cdot r \cdot \overline{Y}/\overline{X} \cdot s_X \cdot s_Y + (\overline{Y}/\overline{X})^2 s_X^2]^{\frac{1}{2}},$$

where s_Y and s_X are defined above. Finally the root mean square of deviations of observations from the regression line was calculated by the expression

$$(5) \qquad \left\{\frac{\Sigma(Y - Y')^2}{n}\right\}^{\frac{1}{2}} = s_Y\{1 - r^2\}^{\frac{1}{2}},$$

where $Y' = a + bX$.

The limits of confidence of the regression line are given by the expression (11)

$$(6) \qquad Y' \pm 2\sigma_{Y'},$$

where

$$(7) \qquad \sigma^2_{Y'} = \sigma^2_Y - Y' \left\{\frac{1}{n} + \frac{(X - \overline{X})^2}{ns_X^2}\right\}.$$

The sample estimate of $\sigma^2 Y - Y'$ is given by

$$(8) \qquad \frac{\Sigma(Y - Y')^2}{n - 2} = \frac{ns_Y^2}{n - 2}\{1 - r^2\}.$$

The sample estimate of σ_Y is therefore given by

$$(9) \qquad \left\{\frac{ns_Y^2}{n - 2} \cdot [1 - r^2] \cdot \left[\frac{1}{n} + \frac{(X - \overline{X})^2}{ns_X^2}\right]\right\}^{\frac{1}{2}}.$$

RESULTS AND ANALYSIS

The individual cardiac outputs obtained with the low-frequency, critically damped ballistocardiograph and certain characteristics of the individuals are presented in Table I. The cardiac outputs determined by the corrected acetylene method will be presented in detail elsewhere (12).

The means and standard deviations of the cardiac outputs and surface areas in the several series of measurements, together with the references, are presented in Table II. Table III presents the correlation coefficients (r) between cardiac output and surface area in the different groups. It will be noted that in the two series of measurements carried out with the high-frequency ballistocardiograph, the value of r is small compared to that obtained when other methods are employed to measure cardiac output.

TABLE II

Cardiac output and surface area data on healthy men

N = number, S.D. = standard deviation

Author	Method	N	Cardiac output, l/min.		Surface area, m^2	
			Mean	S.D.	Mean	S.D.
Starr (13)	B. C. G.*	108	5.73	1.10	1.89	0.13
Tanner (1)	B. C. G.*	50	6.21	0.79	1.85	0.12
Lewis (14)	Acetylene	80	4.07	0.51	1.79	0.14
Grollman (15)	Acetylene	37	4.01	0.35	1.81	0.13
Starr (16)	Ethyl iodide	22	4.47	1.05	1.83	0.13
Selected†	Direct Fick	26	6.39	1.37	1.89	0.16
L. P. H. (12)	Corrected acetylene	34	5.54	0.87	1.89	0.19
L. P. H. (17)	B. C. G.‡	48	6.79	1.43	1.92	0.18

* High-frequency ballistocardiograph.
† Cournand and associates (7), Chapman and associates (4), and Ebert and associates (9).
‡ Low-frequency, critically damped ballistocardiograph.

Neither of the values of r for the high-frequency ballistocardiograph is significantly different from zero and we may conclude that this instrument gives cardiac outputs which do not distinguish between large and small individuals. This is also true of the ethyl iodide method. On the other hand, the direct Fick (catheter), corrected acetylene, low-frequency, critically damped ballistocardiograph and acetylene methods, all yielded values for r which are significantly different from zero. Thus these methods definitely distinguish

TABLE III

Coefficients of correlation between the cardiac output, in liters per minute, and the surface area obtained in the several series presented in Table I, along with the 5 per cent confidence intervals for each value of r

The last column gives the value of r which must be reached if the calculated value of r is considered to be different from zero.

Author	Method	5% confidence values			
		r	Lower	Upper	r at 5% level sig.
Starr	B. C. G. high frequency	0.14	−0.05	0.32	0.20
Tanner	B. C. G. high frequency	0.17	−0.11	0.43	0.28
Lewis	Acetylene	0.50	0.32	0.65	0.22
Grollman	Acetylene	0.53	0.25	0.73	0.33
Starr	Ethyl iodide	0.34	−0.10	0.66	0.42
Selected	Direct Fick, catheter	0.54	0.19	0.77	0.39
L. P. H.	Corrected acetylene	0.60	0.33	0.78	0.34
L. P. H.	B. C. G. low frequency	0.60	0.38	0.76	0.28

TABLE IV

The regression constants relating cardiac output to surface area

The constant b is the slope of the regression line and a is the intercept on the cardiac output (Y) axis.

Method	Regression constants	
	b.	a.
High-frequency ballistocardiograph*	1.12	4.14
Low-frequency ballistocardiograph	4.74	−2.29
Corrected acetylene	2.75	0.34
Direct Fick (catheter)	4.48	−2.11

* Data of Tanner (1)

between large and small individuals. It should be noted that the range of body sizes included in the various samples is roughly the same. The standard deviation of the surface area of Starr's subjects was 0.13. Grollman's subjects had an identical standard deviation for surface area yet in the first case the r relating cardiac output and surface area was 0.14 and in the second 0.53.

On testing the significance of the differences between the several r's presented in Table III, transformed into the z values (10), "student's" t test was used to test the null hypothesis that $z_1 = z_2$ where z_1 and z_2 are the values of the transformed r's under consideration. It was found that the values of the r's obtained with the high-frequency ballistocardiograph differed significantly from those obtained by any of the other methods except that of the ethyl iodide. Tanner (1) has pointed out that the repeatability of the high-frequency ballistocardiograph is high. The test retest correlation coefficient in his series was 0.91. This means that the failure of the high-frequency ballistocardiograph to correlate well with surface area is not due to a large random error in the method. It follows by inference from this that the high-frequency ballistocardiograph is measuring something quite different from that measured by the other methods of determining cardiac output.

Four of the series of cardiac output measurements presented in Tables II and III have been selected for a detailed analysis. The age range of the subjects varied between 18 and 40. The methods and regression constants are presented in Table IV.

The raw data obtained with the low-frequency, critically damped ballistocardiograph are plotted in Figure 1. Both the regression line and the cardiac index line are included in the figure. The cardiac index line was constructed in the usual manner, *i.e.*, the point on the graph given by the mean of the surface area of the group and the

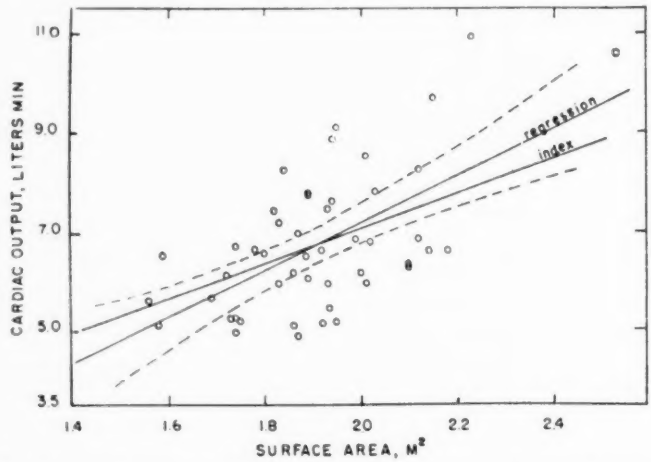

FIG. 1. THE RELATIONSHIP BETWEEN SURFACE AREA OF THE BODY IN SQUARE METERS AND THE BASAL CARDIAC OUTPUT IN LITERS PER MINUTE

The regression line and the cardiac index line are indicated in the figure. The dotted lines are the limits of confidence of the regression line and enclose the range in which will be found the regression lines derived from 95 out of 100 repetitions of this experiment on random samples of this size.

mean of the cardiac output was found and a line was drawn through this point and the zero point. The slope of the regression line and the means of the surface area and cardiac output are subject to sampling error. The possible extent of these errors is indicated by the dotted lines on Figure 1 (11). The range included between these lines includes the regression lines which will be found 95 times in 100 repetitions of this experiment on random samples of this size.

It is apparent that while the regression line is the best possible description of the data at hand, there is a great deal of uncertainty as to the slope of a regression line describing the relationship of surface area and cardiac output of the general population. It is interesting to note that with the exception of the high-frequency ballistocardiograph, the four methods considered in Table IV give regression lines whose limits of confidence enclose the cardiac index line. It is possible, then, that errors in sampling could account for the differences between the cardiac index line and the regression lines of the various samples studied.

To test the relative validity of the linear and the cardiac index methods of prediction, we have elected to use the criteria of accuracy of prediction. This method has been used in the past by Berkson and Boothby (18) to examine the validity of predicting basal metabolism from surface area ratios. Table V gives the root mean square deviations of the observed cardiac outputs minus those calculated by the regression line and the cardiac indices for the four series of measurements. It will be noted that the high-frequency ballistocardiograph gives the largest difference between the root mean squares of the regression line and the cardiac index while the other methods show only small or no difference between the two standards. Comparisons between the absolute magnitudes of the root mean squares of the four methods should be made with caution since multiple determinations for each individual were used to gather data for two of the methods and were not used for the others.

TABLE V

The comparison of the root mean square deviations of observed minus calculated cardiac outputs in liters per minute

	High-frequency B. C. G.*	Low-frequency B. C. G.	Corrected acetylene	Direct Fick
Regression line	0.80	1.14	0.70	1.15
Cardiac index	0.85	1.16	0.70	1.17
Per cent increase	6.3	1.8	0	1.7

* Data of Tanner (1)

TABLE VI

The difference between the cardiac output as predicted by the regression line and that predicted by the index line for different values of surface area

The data for this example are drawn from the series of measurements obtained with the low-frequency ballistocardiograph.

C.O. predicted by \ Surface area, m^2	1.60	1.70	1.80	1.90	2.00	2.10	2.20
Regression	5.29	5.77	6.24	6.72	7.19	7.66	8.14
Cardiac index	5.65	6.00	6.35	6.71	7.06	7.41	7.77
Difference	−0.36	−0.23	−0.11	0.01	0.13	0.25	0.43
Percent of value predicted by reg.	6.8	4.0	1.8	0.2	1.8	3.3	5.3

It is advantageous to compare the variability around the cardiac index line with that around the regression line in terms of the variance (mean sum of squares of deviations) which in this case is simply the square of the figures presented in the body of Table V. This allows us to make proper comparisons of the percentage of excess variability exhibited by the cardiac index over that of the regression standard. Since it can be shown that the variance of the cardiac output from the regression and cardiac index lines are additive, we may write the following expression:

$$V_{CI} = V_R + V_{ex}, \qquad (1)$$

where V is the variance and the subscripts stand for cardiac index, regression and excess, respectively. The percentage of excess variance then becomes

$$P_{ex} = \frac{V_R + V_{ex}}{V_R}. \qquad (2)$$

Applying equation 2, we find that the excess variance of the index line as per cent of the total variance of the regression line is 13.0 per cent in the case of the high-frequency ballistocardiograph, 3.6 per cent for the low-frequency ballistocardiograph, 2.6 per cent for the direct Fick and a negligible amount for the corrected acetylene procedure. Again the high-frequency ballistocardiograph appears to be quite different from the other methods examined here since the use of the cardiac index

TABLE VII

Errors arising from the use of the cardiac index in comparing groups whose mean surface areas are different

The case considered here is that of two groups, both with normal cardiac outputs, drawn from the population described in Figure 1.

Surface area		Cardiac index		Error	Error % of ref. group
Ref. group	Exp. group	Ref. group	Exp. group		
1.90	1.80	3.54	3.46	0.08	2.2
1.90	1.70	3.54	3.39	0.15	4.2
2.00	1.70	3.59	3.39	0.20	5.6
2.10	1.70	3.65	3.39	0.26	7.1
2.20	1.60	3.70	3.30	0.40	10.8

line increases the excess variance by 3.5 times or more than that found with other methods.

To complete the picture, a more detailed description of the errors resulting from the use of the cardiac index in the extremes of body size is useful. An analysis of these errors in the case of the low-frequency ballistocardiograph is presented in Table VI. The errors are a little larger in the case of the small individual as compared to the large and they will not be over 7 per cent in the range of size usually encountered in the northern part of the United States.

Comparisons between the four methods under consideration have been made by examining the figures at one extreme of surface area. For this purpose, the standard deviations of the four series of surface area measurements were averaged and multiplied by 2. This figure was 0.321 square meters of surface area. It was found that in per cent of the value predicted by the regression line at 0.321 square meter above the mean for each group, the error of the cardiac index line was 11.6 per cent for the high-frequency ballistocardiograph, 4.7 per cent for the low-frequency ballistocardiograph, 4.7 per cent for the direct Fick and 0.9 per cent for the corrected acetylene method.

While the error involved in predicting the cardiac output of an individual is small compared to the total variability observed in the cardiac output, the problem of comparing groups is quite different since small differences can be shown to be significant when large groups are involved. Examples of the error arising when two groups whose cardiac output is normal but whose surface area is different are compared and presented in Table VII. It is apparent that if one were interested in comparing a group of very large men with a group of very small men by means of the cardiac index, it would be possible to reach entirely unjustified conclusions.

DISCUSSION

The inference that the high-frequency ballistocardiograph is measuring something only indirectly related to the cardiac output, from the fact that it gives estimates of cardiac output which for all practical purposes are unrelated to body size, has support from other evidence. It has been known for some time that this method gives inaccurate estimates of the cardiac output in the presence of surgical shock (19). Unexplained discrepancies related to the pulse rate in alternative methods of calculating the cardiac output from records of the high-frequency ballistocardiograph have been noted (20). Finally Starr, Horwitz, Maycock and Krumbhaar (21) have recently proposed that the high-frequency ballistocardiograph be used to obtain a measure of cardiac strength, a function which is indirectly related to cardiac output.

Kleiber (22) has pointed out that equations describing physiological data which convey physiological meaning may have value even though in a given sample of the population a more complicated but less meaningful equation would give a better fit to the available data. The cardiac index appears to be a case in point. We have shown that three closely related methods in current use give cardiac outputs which are related to surface area in such a way that the use of the cardiac index adds only a small (less than 3.6 per cent) additional variance over that found by the more efficient least squares method. It should be remembered that even in the best situation the variability attributable to differences in surface area account for only 20 per cent of the total variability observed. The cardiac index is a physiologically meaningful standard which, although somewhat less efficient than the regression line, does not impose an unacceptable penalty when used as a standard to predict the cardiac output of individuals. It is understood that when the cardiac index is used as a standard for individual predictions, it should always be accompanied by precise age specifications since the cardiac index varies with age.

However, when large groups of individuals are to be compared, the data should be examined care-

fully for a possible statistical artifact whose magnitude will depend on the size of the difference in surface area between groups. The solution to this problem is considered to be beyond the scope of this paper and will be considered in detail elsewhere.

It may also be noted that with the available statistical tools, it is not possible to make probability statements as to whether the cardiac index line is actually different from the regression line. The difficulty lies in the fact that we have no knowledge of the sampling differences between slopes of the two equations. If this distribution was available, one could test the hypothesis that the difference between the slopes of the two equations was equal to zero. Then the decision as to whether the cardiac index line is, in fact, a practical method of predicting the cardiac output could be made with confidence corresponding to the probability found.

Many physiologists would insist that the cardiac index line must go through zero. If this is the case and the regression line and cardiac index line are not identical then the cardiac output surface area relationship is not linear but curvilinear. This is an important point since the validity of the above analysis is dependent on the assumption of a linear relationship between the two variables. Unfortunately this question cannot be completely resolved. One can only test for the presence of a curvilinear relationship. On applying the proper statistical test (23), it was found that a definite curvilinear relationship between surface area and cardiac output could not be demonstrated.

SUMMARY AND CONCLUSIONS

1. The relationship between surface area and cardiac output has been examined in a series of observations carried out with the high-frequency ballistocardiograph, the low-frequency, critically damped ballistocardiograph, the ethyl iodide method, the acetylene method, the corrected acetylene method and the direct Fick (catheter) procedure of estimating cardiac output.

2. The high-frequency ballistocardiograph gives estimates of cardiac outputs which are not related to body size (surface area) and is an unsatisfactory method from this point of view. The same is true of the ethyl iodide procedure. The low-frequency, critically damped ballistocardiograph, the acetylene method, the direct Fick (catheter) procedure and the corrected acetylene all distinguish between large and small individuals in a satisfactory manner.

3. The relative efficiencies of the cardiac index and the regression lines as methods for the prediction of the cardiac output from body size (surface area) have been examined in data obtained with four methods for the determination of cardiac output which are in current use.

4. When the cardiac index was used as a standard for data obtained with the low-frequency, critically damped ballistocardiograph, the corrected acetylene and the direct Fick (catheter) procedure, it was found that the variance of the standard increased by only 3.6 per cent or less (less than 2 per cent in terms of the root mean square) over that found when the regression line was used.

5. The limitations of the use of the cardiac index have been discussed.

ACKNOWLEDGMENTS

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SOME STRUCTURAL REQUIREMENTS FOR THE PREVENTION OF LEUKOPENIA INDUCED BY NITROGEN MUSTARD[1, 2]

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Administration of L-cysteine hydrochloride to animals prior to the injection of nitrogen mustard (HN_2) modifies the severe leukopenia characteristically induced by HN_2 (1). However, if the L-cysteine hydrochloride is administered after HN_2, even immediately, the leukopenia is not modified. It has not been demonstrated whether the effect of L-cysteine depends upon the presence of certain groups or groupings in the molecule or whether it is the result of non-specific chemical inactivation of HN_2. Accordingly, the specificity as well as the mechanism of L-cysteine protection was investigated by correlating the chemical structure of related compounds with their ability to prevent HN_2-induced leukopenia.

METHODS

Several amino acids and sulfhydryl-containing compounds structurally closely related to cysteine, as well as compounds containing various combinations of functional sulfhydryl, amino and carboxyl groups were investigated. Ascorbic acid was also tested because, like the other compounds, it is a strong reducing agent.

In addition to L-cysteine, the compounds investigated were glutathione, D,L-homocysteine, D,L-methionine, L-alanine, D,L-serine, thioglycolic acid, dithiopropanol (BAL), thiomalic acid, thiourea, beta mercapto-ethylamine and ascorbic acid (Table I and Figures 1–4).

Except for dithiopropanol (BAL), amounts molecularly equivalent to, or greater than the amounts of L-cysteine which have been found to be effective in preventing HN_2-induced leukopenia in rabbits were used (Table I). Larger amounts of BAL were not used because of their toxicity. Solutions of these substances were adjusted to pH 6.5 to 7.0 with sodium hydroxide and, with the exception of BAL, were injected either intravenously or intraperitoneally. Dithiopropanol (10 per cent solution of BAL in oil) was administered by intramuscular or intraperitoneal injection.

A freshly prepared saline solution of HN_2 [methyl bis (beta chlorethyl) amine hydrochloride[3]] containing 1 mg. per ml. was used in each instance. A single intravenous dose of 2.5 mg. of HN_2 per kg. of body weight uniformly induces a maximal leukopenia in rabbits in four days. The ability of the compounds studied to affect the leukopenia was determined by studying the daily variations in the total and differential leukocyte counts when these compounds were administered 10 to 15 minutes prior to the injection of HN_2.

TABLE I

Prevention of leukopenia induced by nitrogen mustard

Compound investigated	Incubation with nitrogen mustard prior to injection		Compound administered prior to injection of nitrogen mustard	
	No. of rabbits	Maximum fall in WBCs (% of control)	No. of rabbits	Maximum fall in WBCs (% of control)
L-cysteine hydrochloride 150–1,000 mg. per kg.	7	16.6	20	37.5
Glutathione 1,250–1,500 mg. per kg.	5	30.7	6	61.8
D,L-homocysteine 300–500 mg. per kg.	—	—	7	65.3
D,L-methionine 650–750 mg. per kg.	5*	0	5*	87.0
D,L-alanine 500–1,000 mg. per kg.	5	0	12	80.8
D,L-serine 900–1,200 mg. per kg.	5*	23.0	5*	81.3
Thioglycolic acid 400–500 mg. per kg.	5	14.5	7	90.3
Dithiopropanol (BAL) 50 mg. per kg.	5*	26.6	8†	88.2
Thiomalic acid 600 mg. per kg.	3	0	4	79.2
Thiourea 400 mg. per kg.	3	36.6	5	78.7
Beta mercapto-ethylamine 200 mg. per kg.	—	—	2	77.7
Ascorbic acid 100 mg. per kg.	6	79.7	5	80.1
Nitrogen mustard 2.5 mg. per kg.	5‡	85.3	21	79.9

* Administered intraperitoneally.
† Administered intramuscularly.
‡ Methyl bis (beta chlorethyl) amine hydrochloride incubated for 30 minutes at 37° C.

The effect of direct chemical interaction with HN_2 was studied by incubating the compounds under investigation with HN_2 *in vitro* at 37° C. for 30 minutes in the same

[1] This study was made possible by a grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

[2] Presented in part at the Central Society for Clinical Research, Chicago, November 2, 1951.

[3] The nitrogen mustard and L-cysteine hydrochloride used in these experiments were furnished through the courtesy of Merck & Company, Rahway, New Jersey.

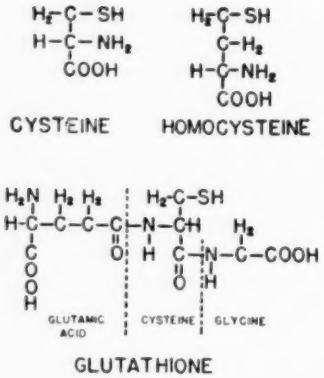


FIG. 1.

These compounds having vicinal sulfhydryl, amino and carboxyl groups are capable of modifying the leukopenia induced by nitrogen mustard. Substitution or alteration of either the free sulfhydryl, amino or carboxyl group results in the complete disappearance of any detectable protective effect against mustard-induced leukopenia.

amounts as were used *in vivo*. The resultant mixtures were then injected into rabbits and the changes in leukocyte count were determined. These changes were compared with the effect obtained when the same amounts of these compounds were given parenterally 10 to 15 minutes prior to the administration of the same amounts of HN_2.

RESULTS

With the exception of ascorbic acid, injection of incubated mixtures of HN_2 and the various compounds caused very little leukopenia compared to

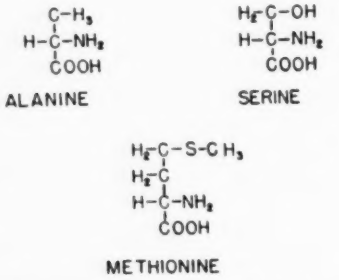


FIG. 2. COMPOUNDS CONTAINING A FREE AMINO AND VICINAL CARBOXYL GROUP

Although these compounds are structurally closely related to cysteine, they lack a free sulfhydryl group and exert no protective effect against mustard-induced leukopenia.

that which would have been produced by the same amount of HN_2 alone. The maximum decrease in the leukocyte count following the injection of a saline solution of HN_2 which had been incubated by itself for 30 minutes at 37° C. was 85.3 per cent of the control count. Administration of the same dose of HN_2 following incubation with equivalent amounts of L-cysteine, glutathione, D,L-methionine, D,L-alanine, D,L-serine, thioglycolic acid, BAL, thiomalic acid, and thiourea resulted in a decrease in the leukocyte count ranging from 0 to 36.6 per cent of the control counts (Table I, Figure 5). The differential leukocyte count did not change. There was no significant decrease in the degree of leukopenia induced by HN_2 following

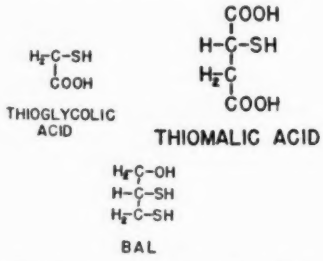


FIG. 3. COMPOUNDS CONTAINING FREE SULFHYDRYL GROUPS

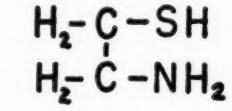
Although some of these compounds contain a carboxyl group in addition to the sulfhydryl group, they do not contain an amino group. These compounds fail to prevent the mustard-induced leukopenia. The presence of two free sulfhydryl groups in BAL does not result in protection against the leukopenia induced by nitrogen mustard.

its incubation with ascorbic acid. Chemical interaction between HN_2 and most of the compounds studied did not occur at room temperature, but only after incubation at 37° C. for 30 minutes.

In contrast to the results obtained after administration of the incubated mixtures were those obtained when equivalent amounts of the different compounds were injected prior to the HN_2. Only L-cysteine and to a much lesser extent D,L-homocysteine and glutathione were capable of modifying the leukopenia (Table I, Figure 6). The average maximum fall in the leukocyte count induced by HN_2 was 79.9 per cent of the control counts. When L-cysteine was injected prior to HN_2, the average maximum fall in the leukocyte

count was 37.5 per cent of the control counts. Injection of equivalent amounts of glutathione and D,L-homocysteine prior to HN_2 resulted in an average maximum fall in the leukocyte count of 61.8 and 65.3 per cent of the control counts, respectively.

Injection of equivalent amounts of the other compounds prior to the injection of HN_2 resulted in a maximum fall in the leukocyte count ranging from 78.7 to 90.3 per cent of the control counts, compared with the fall of 79.9 per cent with HN_2 alone. The most severe leukopenia occurred when either thioglycolic acid, BAL, or methionine was injected prior to HN_2 (Table I). The maximum leukopenia invariably occurred on the fourth or fifth day following injection.



BETA MERCAPTO ETHYLAMINE

FIG. 4. COMPOUND CONTAINING A FREE SULFHYDRYL AND AMINO GROUP WITHOUT A CARBOXYL GROUP

Although this compound has the same structural configuration as cysteine except for the absence of a carboxyl group, it does not prevent mustard-induced leukopenia.

Injection of the compounds without subsequent administration of HN_2 did not cause a significant change in the leukocyte counts.

DISCUSSION

It is apparent that many highly reactive compounds with structures closely related to cysteine do not modify the leukopenia induced by HN_2. The modification of mustard-induced leukopenia appears to depend upon the presence of certain specific groups in close apposition in the molecule. An analysis of some of the structural requirements reveals the following:

1. The role of vicinal sulfhydryl, amino and carboxyl groups: A comparison of the structure of the compounds studied with the ability to pre-

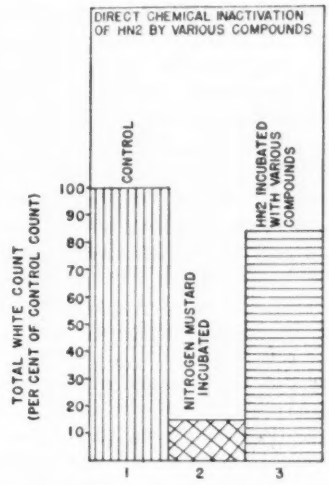


FIG. 5. AVERAGE LEUKOCYTE COUNTS OBTAINED WHEN NITROGEN MUSTARD IS INCUBATED *IN VITRO* WITH VARIOUS COMPOUNDS PRIOR TO INJECTION

L-Cysteine, glutathione, methionine, alanine, serine, thioglycolic acid, BAL, thiomalic acid and thiourea inactivate mustard when allowed to react directly with HN_2 prior to administration. However, only L-cysteine, glutathione and D,L-homocysteine modify mustard-induced leukopenia when injected *in vivo*.

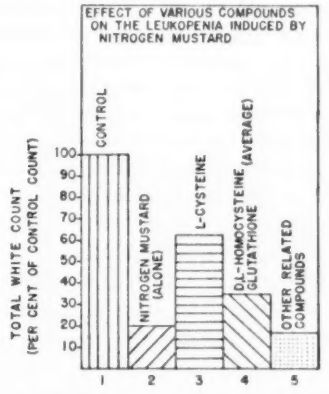


FIG. 6. AVERAGE LEUKOCYTE COUNTS OBTAINED WHEN NITROGEN MUSTARD IS INJECTED AFTER THE ADMINISTRATION OF VARIOUS COMPOUNDS

L-Cysteine is the most effective in preventing leukopenia (column 3). D,L-homocysteine and glutathione are not as effective (column 4). Methionine, alanine, serine, thiomalic acid, thioglycolic acid, BAL, thiourea and ascorbic acid are completely ineffective in modifying the mustard-induced leukopenia (column 5).

vent leukopenia reveals that only those compounds which have a sulfhydryl, amino and carboxyl group in close apposition were capable of preventing HN_2-induced leukopenia (Figure 1). Thus, L-cysteine, D,L-homocysteine and glutathione had a definite protective effect. L-Cysteine, which has a carboxyl group, a free sulfhydryl group, and an amino group on adjacent carbon atoms, was by far the most effective. D,L-Homocysteine, in which the free sulfhydryl and free amino group are separated by one carbon atom, was much less effective than L-cysteine. It is possible that one of the optical isomers of homocysteine may not be effective in preventing leukopenia and that the decreased protection was due to the presence of an inactive isomer in the D,L mixture of the amino acid. Methylation of the sulfhydryl group in D,L-homocysteine to form D,L-methionine (Figure 2) resulted in the complete disappearance of any detectable protective effect.

Glutathione is composed of one molecule of glutamic acid, one of cysteine, and one of glycine (Figure 1). The partial prevention of HN_2-induced leukopenia by glutathione may be attributed to the presence of cysteine in the molecule. Although the amount of glutathione injected contained sufficient cysteine (as a constituent of glutathione) to furnish satisfactory protection, the prevention of leukopenia was much less than that obtained with equivalent amounts of L-cysteine. This may indicate that the conjugation of the amino or carboxyl group, or both, in cysteine to form glutathione resulted in decreased ability to protect against leukopenia, or that the release of cysteine from glutathione *in vivo* was too slow to furnish optimum protection.

2. The effect of compounds containing vicinal amino and carboxyl groups without an adjacent sulfhydryl group: The injection of compounds containing a free amino and vicinal carboxyl group without a sulfhydryl group resulted in the absence of any protective effect. Thus, the replacement of the sulfhydryl group in cysteine with an hydroxyl group to form serine or with hydrogen to form alanine (Figure 2) resulted in complete failure of protection. Thiourea ($NH_2 \cdot CS \cdot NH_2$), containing two amino groups without vicinal carboxyl or sulfhydryl groups, was likewise ineffective.

3. The role of free sulfhydryl groups: Compounds containing a free sulfhydryl group without an amino group in close proximity were ineffective also, even though one or two carboxyl groups were adjacent (thioglycolic and thiomalic acid, Figure 3). The failure of BAL, which has two vicinal sulfhydryl groups, to manifest a protective effect is further evidence that the presence in itself of sulfhydryl groups is not sufficient to prevent leukopenia.

4. The role of the carboxyl group: The presence of a carboxyl group in addition to vicinal amino and sulfhydryl groups is essential for a protective effect. Thus beta mercaptoethylamine [4] which has the same structural configuration as cysteine without a carboxyl group (Figure 4) does not modify HN_2-induced leukopenia. This compound was very toxic, several animals dying within three to four hours following intravenous or intraperitoneal administration. The cause of death in these animals is unknown. No irritation or adverse reaction was noted during the period of injection nor immediately after.

Thus under the conditions of these experiments, neither the reducing potential of a compound, nor the presence by themselves of free carboxyl, sulfhydryl, or amino groups could be correlated with the ability of the compounds studied to prevent leukopenia when injected prior to HN_2. Those compounds exhibiting a protective effect uniformly have in common the presence of a closely apposed free sulfhydryl, amino and carboxyl group.

The mechanism by which this specific structural configuration results in modification of the HN_2-induced leukopenia is not known. The lack of sparing effect of closely related compounds could conceivably indicate only that the affinity of these compounds for HN_2 *in vivo* is not as great as that of L-cysteine, D,L-homocysteine and glutathione; even though they are able to combine with HN_2 *in vitro*. It is possible that the presence of vicinal sulfhydryl, amino and carboxyl groups permit intracellular penetration thereby enabling these compounds to inactivate HN_2. However, L-cysteine likewise modified the leukopenia induced by irradiation (2) where chemical inactivation of

[4] The beta mercaptoethylamine used in these experiments was prepared and furnished through the courtesy of Dr. Sam Seifter, State University of New York, College of Medicine at New York City.

noxious substances is presumably not involved. It is possible, therefore, that L-cysteine protection of leukocytes against damage by HN_2, as with irradiation, is not due solely to chemical inactivation.

Fruton and associates (3, 4) have shown that HN_2 combines *in vitro* with the functional groups of many proteins and important biologic substances. They suggested that HN_2 may produce its effects by inactivating similar substances *in vivo*. It may then be postulated that HN_2-induced leukopenia results from the inactivation or destruction of some of these substance(s) by HN_2 which are essential for normal leukopoiesis, and that L-cysteine or related compounds exert a sparing or protective effect by virtue of competition with HN_2.

There is abundant evidence that sulfhydryl-containing compounds are involved in leukopoiesis. It is known, for example, that sulfhydryl compounds are concerned with cell division and growth, that the sulfhydryl content of immature cells is greater than that of mature cells and that the blood sulfhydryl content is low in hypoplastic states (5). Furthermore, irradiation and drugs that produce a granulocytopenia also produce a fall in the blood sulfhydryl level. A specific molecular configuration involving sulfhydryl, amino and carboxyl groups may be involved in these phenomena. Thus L-cysteine and related compounds may have a role in leukocyte formation or in abnormal leukopoiesis. Further investigation of the role of cysteine and cysteine analogues in leukocyte metabolism is in progress.

The effect of D-cysteine as compared with L-cysteine was not investigated because of the unavailability of D-cysteine. It has been shown (6), however, that L-cysteine can reverse cysteine deficiency produced in rats fed low cysteine diets containing bromobenzene, whereas D-cysteine is ineffective. If D-cysteine were similarly ineffective in preventing the leukopenia induced by HN_2, it would be further evidence that the mechanism of the L-cysteine protection is other than that solely of chemical inactivation of HN_2 *in vivo*.

The failure of HN_2 to produce leukopenia when injected after incubation *in vitro* with most of the compounds studied, indicates that the HN_2 was inactivated by this process. HN_2 undergoes rapid intramolecular rearrangement in aqueous solution to form an imine linkage (7). In this form it is highly reactive and is capable of alkylating free sulfhydryl, amino and carboxyl groups (3). It is likely that alkylation of these groups in the compounds studied was permitted to take place during direct chemical interaction *in vitro*, thereby resulting in loss of potency of the HN_2. While the reaction efficiency of these compounds was not determined, the protective effect of L-cysteine against leukopenia induced by irradiation indicates that factors such as blood level and solubility may not be involved in the failure of compounds with closely related structure to prevent leukopenia.

SUMMARY AND CONCLUSIONS

1. The specificity and mechanism of L-cysteine protection against HN_2-induced leukopenia were investigated by studying the relationship of structure to ability to prevent leukopenia.

2. Of the compounds studied, only those with vicinal sulfhydryl, amino, and carboxyl groups were capable of modifying HN_2-induced leukopenia. L-Cysteine, which has a free sulfhydryl, amino and carboxyl group on adjacent carbon atoms was the most effective in preventing leukopenia. D,L-Homocysteine and glutathione were less effective.

3. Substitution or alteration of either the free sulfhydryl, amino or carboxyl group results in the complete disappearance of any detectable protective effect against HN_2-induced leukopenia.

4. Under the conditions of this experiment, neither the reducing action of a compound, nor the presence by themselves of free sulfhydryl, amino or carboxyl groups can be correlated with the ability of the compounds studied to prevent leukopenia when injected prior to nitrogen mustard.

5. The data indicate that the protective effect of L-cysteine and its homologues *in vivo* does not result solely from inactivation of HN_2. It is more likely that L-cysteine protects some substance(s), essential for leukopoiesis, from destruction by HN_2.

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AN IMMUNOCHEMICAL STUDY OF THE ALBUMINS OF SERUM, URINE, ASCITIC FLUID AND EDEMA FLUID IN THE NEPHROTIC SYNDROME [1, 2]

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The serum and urinary proteins in the nephrotic syndrome have been studied by a number of investigators and the divergence of results has given rise to conflicting interpretations concerning the pathogenesis of this disease. With but few exceptions (1, 2), there appears to be general agreement that the urinary proteins are derived from the plasma. This was suggested by qualitative immunological tests by Mertens (3), Erben (4), and Müller (5), by optical rotation by Hewitt (6), by racemization by Cavett and Gibson (7), and by a variety of physical-chemical methods by Widdowson (8).

On the other hand, comparisons between the serum proteins of these patients and the serum proteins of normal individuals have indicated marked differences. Goettsch and Reeves (9) studied the serum and urinary proteins in nephrosis through the use of quantitative immuno-chemical procedures, and concluded that the serum proteins in this disease differed immunologically from the normal; in their method, they compared protein levels derived immunochemically with those derived by salt fractionation, the latter being used as the reference standard. Alving and Mirsky (10) found that the albumin fraction separated with ammonium sulfate had a lower cystine content than did similar fractions of normal serum, but upon further purification, the resulting nephrotic serum albumin had the same cystine content as normal serum albumin. Albanese, Davis, Smetak and Lein (11), using ammonium sulfate fractions, found an abnormal distribution of amino acids in the plasma proteins of nephrotic patients.

With the introduction of the Tiselius apparatus, the Howe method for the separation of albumin and globulins in serum and urine was shown to be in serious error, particularly where the proportion of albumin was low (12). Longsworth and MacInnes (13) and Luetscher (12) showed that the albumin content was very low in nephrotic serum and that the pattern of the urinary proteins in the nephrotic syndrome resembled that of normal serum. These findings would serve to explain many of the abnormalities of the "albumins" obtained by salt fractionation of nephrotic serum. However, Luetscher did observe that in nephrotic serum and urine the ratio of the two components of albumin separated by electrophoresis at pH 4.0 was reversed from the "normal" and he postulated an abnormality in the metabolism of albumin in this syndrome.

The present study is an attempt to compare by immunochemical characterization the albumins of the plasma, urine, ascitic and edema fluids of nephrotic patients with crystallized albumin obtained from the pooled plasma of normal humans (14, 15).

MATERIALS AND METHODS

Patients: Eleven children with the nephrotic syndrome were selected for study. The pertinent aspects of their disease are presented in Table I. Their ages ranged from 19 months to 5.3 years, and the duration of illness from the time of onset to the time of study was from two months to 24 months. Serum was obtained from each of the 11 patients, ascitic fluid from eight patients and an additional patient, D. D. R., urine from six and edema fluid from two. Serum was also obtained from a 12th child, R. S., who had chronic glomerulonephritis with hypertension and congestive failure with anasarca.

Antisera: The antigen employed throughout this study was crystallized human serum albumin, preparation Decanol 10 (15), crystallized from fraction V obtained from the pooled plasma of normal adults by low-temperature ethanol-water fractionation (14). One group of five rabbits was given three subcutaneous injections of crystal-

[1] Presented before the Society for Pediatric Research at Atlantic City, May 2, 1951.

2. Supported by grants from the National Institutes of Health, U. S. Public Health Service.

[3] Postdoctorate Research Fellow of the National Institutes of Health.

lized human serum albumin per week for four weeks, 15 mg. per kilo body weight per injection, and then bled by cardiac puncture 10 days after the last injection. A second group of six rabbits was given an albumin-aluminum hydroxide suspension subcutaneously, one injection of 25 mg. albumin per kilo body weight every 12 days for four injections, and these animals were bled by cardiac puncture 14 days after the last injection. The albumin-aluminum hydroxide suspension was prepared by dissolving 1.25 gm. crystallized human serum albumin in 5 ml. saline and then adding this solution to 45 ml. washed aluminum hydroxide gel.[4] The gel was washed prior to the addition of the albumin by adding to it an equal volume of 0.15 M saline, allowing the gel to sediment overnight, decanting and rewashing the precipitate with 0.15 M saline once again. The sera of the animals within each group were pooled and the sera stored at −30° C. until use.

The preparation of the horse antiserum *vs.* human serum albumin, used in this study, has been described (16). The crystallized human serum albumin employed as an antigen in the horse was the same preparation used for immunizing the rabbits.

All of the antisera were found to be specific for human serum albumin and to contain no detectable precipitins for γ-globulins, β-lipoprotein, or β_1-metal combining globulin obtained from human plasma. In addition, after adsorption with crystallized human serum albumin, these antisera did not precipitate with any dilution of whole human serum, again demonstrating the probable absence of precipitating antibody for any protein of human plasma other than albumin.

Immunochemical analysis of body fluids: Each of the body fluids studied was appropriately diluted with 0.15 M sodium chloride solution, so that a series of antigen concentrations was obtained that would cover all the zones of inhibition and precipitation when aliquots were reacted with the rabbit and horse antisera employed. Antigen-rabbit antiserum reaction mixtures were prepared by mixing 0.5 ml. of each of the serial dilutions of a given body fluid sample with 1.0 ml. aliquots of rabbit antiserum. The mixtures were incubated at 37° C. for one hour, then 1° C. for 24 hours. The precipitate was washed twice with cold 0.15 M saline, employing centrifugation at 4,000 r.p.m. at 0° C. for 20 minutes to recover the precipitate. The amount of specific precipitate was measured by the spectrophotometric method (17).

In using the horse antiserum system, 1.0 ml. aliquots of suitable dilutions of the given sample of body fluid to be studied were mixed with 1.0 ml. aliquots of horse antiserum, the mixtures incubated at 37° C. for one hour and then at 0° C. for three days. The precipitate was recovered by centrifugation at 0° C. at 4,000 r.p.m. for 30 minutes, washed twice with cold saline, and measured spectrophotometrically.

[4] The aluminum hydroxide gel employed was Creamalin®, a suspension prepared by Winthrop-Stearns Company.

All antisera were clarified immediately before use by filtration through an asbestos filtering pad. Appropriate dilutions of crystallized human serum albumin in 0.15 M saline were employed as standards for obtaining the standard antigen-antibody reaction curves. The final volume of the reaction mixture and the amount of antiserum in the mixture were kept constant and the amount of antigen added to the system in a constant volume was varied.

The antigen-antibody curves were constructed in the conventional manner. The amount of antigen added to a constant volume of antiserum was plotted along the abscissa and the amount of specific precipitate obtained from the antigen-antiserum mixture was plotted, as optical density, along the ordinate. Since there was no method available for the accurate[5] estimation of albumin in body fluids where the proportion of albumin is low such as nephrotic serum, edema fluid and ascitic fluid, the absolute amount of antigen in these body fluids added to the antiserum, and hence the absolute units employed along the abscissa, could not be ascertained. Therefore, the amount of antigen in the nephrotic fluid studied was plotted on a *relative* scale along the abscissa, selecting as unity that amount of nephrotic fluid giving a specified quantity of specific precipitate, in terms of optical density, with the rabbit antiserum. The specified optical density selected as the reference point was held constant for all curves including that obtained with crystallized normal serum albumin and thus all curves with a given rabbit antiserum were adjusted to the same scale. In all the figures presented the relative scale has been reconverted to mg. of albumin, since the absolute amounts of crystallized human serum albumin added to the reaction mixtures to obtain the standard curves were known.

The assumptions inherent in such a procedure are first, that antigens giving superimposable precipitation curves with a given antiserum under the conditions of these experiments are immunologically identical, and second, that antigens giving precipitation curves which differ in shape from those of the standard must differ from it im-

[5] The error in estimation of albumin in these fluids would have to be less than about 5% (of the amount of albumin present) if any significance were to be attached to the immunochemical results. The only method approaching this accuracy at low albumin concentrations at this point in the investigation was electrophoresis; the statistical error in the interpretation of the electrophoretic diagram would be about 1% but at albumin levels of about 10%. This would mean a 10% error in the estimation of the amount of albumin present. The error due to interference by α and β globulins would also be significant (18). In addition, the absolute amount of total protein present would have to be measured and any method adopted would be subject to serious error due to the high lipid content of all the sera studied and the abnormal ratio of the protein components (14, 19). This error coupled with the errors in interpretation of the electrophoretic pattern would make this procedure hazardous for absolute values at low albumin: total protein ratios.

TABLE I

Patient*	Age (years)	NPN† (mg. %)	Blood pressure	Gross‡ hematuria	Duration of illness (months)	Concentration§ of albumin in		
						Serum (gm. %)	Ascitic fluid (gm. %)	Edema fluid (gm. %)
J. R.	2 11/12	20–47	106/64	0	15	0.60	0.023	
J. S. M.	2 8/12	21–54	106/68	0	13	0.28	0.006	
C. H.	3 10/12	31–41	110/70	0	9	0.32	0.009	0.001
K. P.	4 4/12	30–60	104/70	+	13	0.29	0.012	
M. P.	4 5/12	26–49	110/70	0	24	0.42		
J. F.	2 2/12	33–52	108/72	0	9	0.16	0.005	
W. G.	5 4/12	36–65	100/60	0	11	0.09	0.005	
L. N.	4 8/12	46–104	106/70	+	24	0.46	0.044	0.003
R. O.	3 1/12	30–37	114/76	0	2	0.27	0.006	
B. P.	1 7/12	32–34	108/70	0	3	0.22		
D. D. R.‖	1 11/12	28–39	110/70	0	5		0.018	
J. K.	2 5/12		90/48	0	12	0.51		
R. S.¶	7 11/12	116–155	150/112	0	18	1.57		

* All patients had anasarca and ascites at time of study.
† Range during illness.
‡ Microscopic hematuria to some extent was observed in most patients at some time during their illness.
§ As determined immunochemically employing rabbit antiserum. Specimens of serum, and of ascitic and edema fluids were not necessarily obtained simultaneously.
‖ Only ascitic fluid was studied in D. D. R.
¶ R. S. is a patient with chronic glomerulonephritis, hypertension and congestive heart failure.

munochemically, at least in part. These assumptions seem to be justified by immunological experience with other antigen-antibody systems. In the case of human serum albumins which have been markedly altered by conjugation with azodyes, we have shown (20) that these modified proteins will still precipitate with antisera against human serum albumin but with a different precipitation curve from that obtained with the native protein.

In those instances where the proportion of albumin was known to be as high as 85% to 95% of the proteins present, as in the urine protein samples, the percentage of albumin was determined electrophoretically; the total protein was calculated by dialyzing the fractionated urinary proteins against saline and then saline-buffer (phosphate buffer, pH 7.3, $\Gamma/2 = 0.05$; NaCl, $\Gamma/2 = 0.10$) and determining the difference in the dry weights of aliquots of the fluid inside and outside the membrane. In the case of the urine samples, therefore, the figures along the abscissa represent the actual amount of albumin as calculated for both the albumin standards and the urinary proteins.

Preparation of materials for analysis: Since the protein content of edema fluid and ascitic fluid proved too low to permit adequate characterization, these fluids were concentrated 10 to 30-fold by placing them in long lengths of narrow dialyzing tubing and suspending the tubing at 1° C. for four to seven days. The concentrated fluids were then dialyzed against 0.15 M saline and finally, several changes of saline-buffer.

To prevent denaturation of the albumin in urine by its interaction with other urinary constituents, the urinary proteins were rapidly separated from the urine by precipitation with ethanol at low temperatures employing Method 10 (21) with slight modifications.[6] The urinary albumin was extracted from the protein precipitate with 19% ethanol at pH 5.8; after several precipitations and extractions, the albumin fraction was lyophilized.

Normal human serum was rendered low in its proportion of albumin relative to the other serum proteins by precipitation and extraction with alcohol by Method 10 (21), the reconstituted serum representing a solution of the initial precipitate of this technique. Albumin separated from the albumin-rich supernatant of this fractionation procedure was found to be identical both immunochemically and physico-chemically with the crystallized albumin employed as an antigen in these studies, thus making it unlikely that specific albumin components rather than the major portion of the total albumin had been removed from these normal sera. Electrophoretic analyses of normal sera so prepared revealed low albumin: total protein ratios very similar to those of sera from nephrotic patients. Precipitation curves of rabbit and horse antisera were obtained with these low-albumin "normal" sera as controls for possible interference in the precipitation of albumin with either type of antibody by specific or non-specific interaction of albumin with other proteins in human serum.

RESULTS

The results are given in a series of figures which compare the experimental points obtained with various materials with the standard curve for crystallized normal human serum albumin. In

[6] With the collaboration of Dr. Lewis Barness.

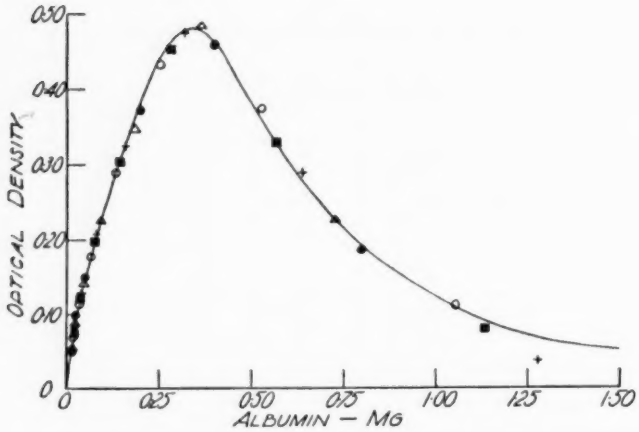

FIG. 1. THE PRECIPITATION CURVES OBTAINED IN THE REACTION BETWEEN A GIVEN RABBIT ANTISERUM VS. NORMAL HUMAN SERUM ALBUMIN AND SEVERAL NEPHROTIC SERA

In this figure and in all subsequent figures, the solid line represents the precipitation curve obtained in the reaction between the antiserum and crystallized normal human serum albumin, and the symbols represent values for the specimen from a particular patient.

○ M. P. △ K. P.
● C. H. ■ L. N.
\+ J. K.

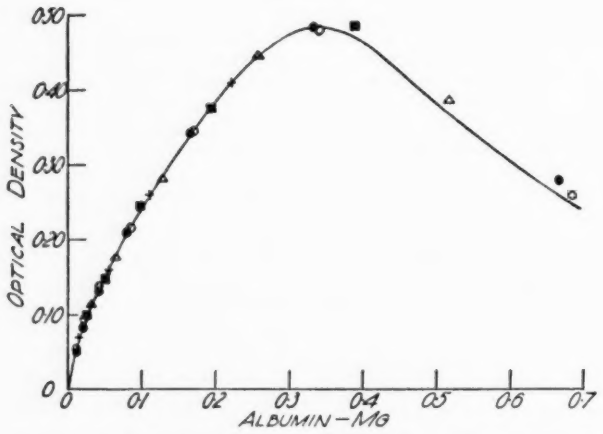

FIG. 2. THE PRECIPITATION CURVES OBTAINED IN THE REACTION BETWEEN A GIVEN RABBIT ANTISERUM VS. NORMAL HUMAN SERUM ALBUMIN AND SEVERAL NEPHROTIC SERA

In the case of J. F., a paracentesis was performed and the patient lost most of his edema fluid between the time of the first and last specimens studied.

■ J. F. 4/26/50 ● R. O.
△ J. F. 4/27/50 \+ W. G.
○ J. F. 4/29/50

all these figures the solid line indicates the standard curve and the symbols represent the experimental points obtained and plotted as described above, each symbol representing material from a particular patient. Estimated concentrations of albumin in various fluids obtained from different patients are reported in Table I.

A. Characterization with Rabbit Antisera

1. Albumins of serum. The precipitation curves obtained by reacting the rabbit antisera with nephrotic sera were indistinguishable over all zones of precipitation and inhibition from those obtained

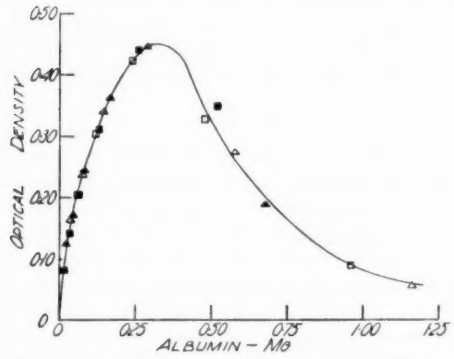

FIG. 3. THE PRECIPITATION CURVES OBTAINED IN THE REACTION BETWEEN A GIVEN RABBIT ANTISERUM VS. NORMAL HUMAN SERUM ALBUMIN AND SEVERAL NEPHROTIC SERA

Included is the precipitation curve obtained with the serum of a patient with severe glomerulonephritis, R. S.

△ J. R. □ R. S.
▲ J. S. M. ■ B. P.

when reacting the same antiserum with its homologous antigen, crystallized human serum albumin, as shown in Figures 1 to 3. Serum samples were obtained from a single patient, J. F., over a period of several days during which a paracentesis was performed and was followed by drainage through the paracentesis wound, loss of all edema, and a rise in serum albumin from 0.16 gm.% to 0.28 gm.% in three days. The albumins in the three sera from this patient were identical immunochemically, as illustrated in Figure 2. Patient R. S. had chronic glomerulonephritis, hypertension with congestive failure, and a substantially higher

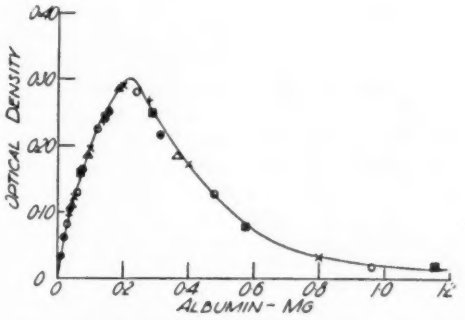

FIG. 4. THE PRECIPITATION CURVES OBTAINED WITH A GIVEN RABBIT ANTISERUM AND SEVERAL ASCITIC FLUIDS, AN EDEMA FLUID AND A NORMAL SERUM RENDERED LOW IN ALBUMIN CONTENT

Ascitic fluids: ○ D. D. R. + J. S. M.
● R. O. ⊠ J. R.
Edema fluid: △ C. H.
Low albumin, "normal serum No. 1": ×

serum albumin level (1.57%) than those of the other patients, but the points determined for his serum albumin fell on the standard curve (see Figure 3).

2. Albumins of ascitic fluid and subcutaneous edema fluid. The precipitation curves for these albumins are shown in Figure 4. They are identical with the standard curve obtained with crystallized normal human serum albumin.

3. Albumins of urine. The urinary albumin

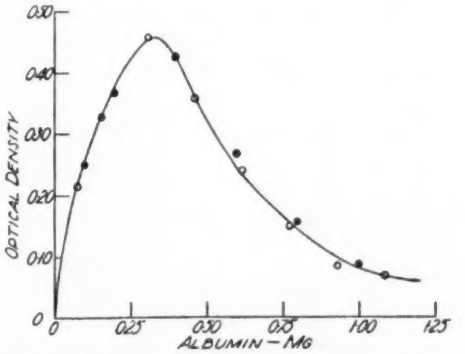

FIG. 5. THE PRECIPITATION CURVES OBTAINED IN THE REACTION BETWEEN A GIVEN RABBIT ANTISERUM VS. NORMAL HUMAN SERUM ALBUMIN AND TWO SAMPLES OF URINARY ALBUMIN

○ J. S. M.
● C. H.

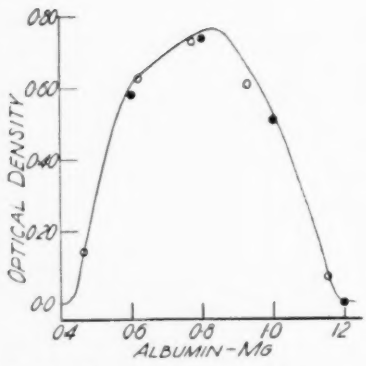

FIG. 6. THE PRECIPITATION CURVES OBTAINED IN THE REACTION BETWEEN HORSE ANTI-ALBUMIN AND THE TWO SAMPLES OF URINARY ALBUMIN OF FIGURE 5

curves were identical with those obtained with normal serum albumin when reacted with rabbit antisera, as shown in Figure 5. It should be noted that the amount of urinary albumin added to the antiserum was calculated from electrophoretic and dry weight data and the abscissae in this figure, therefore, represent absolute values.

B. Characterization with Horse Antiserum

The curves obtained with two nephrotic urinary albumins were identical with the standard curve using horse antiserum as shown in Figure 6.

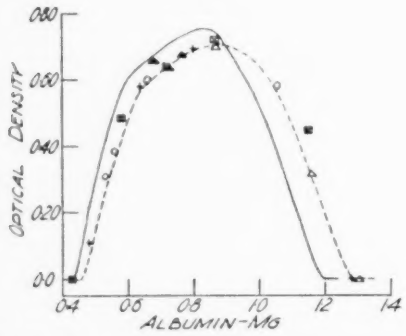

FIG. 7. THE PRECIPITATION CURVES OBTAINED IN THE REACTION BETWEEN HORSE ANTI-ALBUMIN AND SEVERAL NEPHROTIC FLUIDS

Sera: ○ M. P. △ J. R.
+ J. K. ▲ J. S. M.
Ascitic fluid: ⊠ J. R.
Low albumin, "normal serum No. 1": dashed line.

However, serum and ascitic fluid albumins in the human albumin-horse anti-albumin system gave slightly different curves from those obtained with crystallized normal serum albumin, as illustrated in Figure 7. It is important to note at this time that the albumin contents of these nephrotic fluids were estimated from the rabbit antiserum curves (17) and plotted as "absolute" values along the abscissa for the horse antiserum system. The horse serum curves for nephrotic serum and ascitic fluid albumins were identical, however, with those obtained by reacting the horse antiserum with low-albumin "normal" human serum as indicated in Figure 7. To keep the procedure uniform, the amount of albumin in the low-albumin "normal" serum used along the abscissa in Figure 7 was estimated by the rabbit antiserum method and the rabbit curve for this serum is shown in Figure 4.

DISCUSSION

From the data presented, there does not appear to be any immunochemical abnormality of the circulating serum albumin or of the albumin found in the edema fluid, ascitic fluid or urine of these patients. The thesis, therefore, that albuminuria in the nephrotic syndrome represents the excretion (11) or retention (9) of abnormal circulating serum albumin is not substantiated by the data reported here. It would appear instead that the albuminuria is related primarily to the pathological defect in the kidney. This is in accord with the finding that patients with the nephrotic syndrome retain only a small portion of intravenously injected albumin prepared from pooled normal plasma (22).

The low albumin levels we have found in the sera of patients with the nephrotic syndrome during the edematous phases of this disease agree well with those reported in the electrophoretic studies of Longsworth and MacInnes (13) and Luetscher (12). The higher serum albumin level found in the single patient with severe chronic glomerulonephritis is in accord with the findings of Heymann and Lund (23): in experimental nephrotic syndome in rats, they found marked hypoproteinemia in the acute phase, but gradual improvement in this finding occurred as the disease progressed.

The simultaneous precipitin studies with rabbit and horse antisera were interesting in that the horse system appeared to measure less albumin

in a given sample than did the rabbit serum. At least three explanations seem possible. It may be postulated that the horse and rabbit antisera react differently with different albumin components and, therefore, human serum albumin is immunochemically heterogeneous. That crystallized normal human serum albumin is physico-chemically heterogeneous is beyond question (15). However, since crystallized normal serum albumin was used for the standard curves for both the rabbit and horse systems, one must also postulate that the *ratio* of immunochemical components in the crystallized normal serum albumin differed from the ratio of these components in both normal and nephrotic sera. An alternative hypothesis is that serum albumin *in its native state* interacts with other serum proteins sufficiently to interfere with its precipitation by horse antiserum but not by rabbit antiserum. Interaction among the serum proteins has been repeatedly demonstrated. That the rabbit and horse systems differ in their reactivity with human serum albumin has also been demonstrated (20). A third possibility is that the rabbit antisera interacted with another plasma protein in addition to albumin. However, precipitins for plasma proteins other than albumin could not be demonstrated in the antisera used.

The albumin concentrations obtained with the horse antiserum system as estimated from the precipitation curves were about 5% to 10% lower than those obtained with the rabbit system. When the albumin levels being measured are considered, it appears that the horse system is as reasonably accurate as electrophoresis for the estimation of serum albumin at low levels and hence substantiates earlier findings (16).

It should be pointed out that the antisera used were all prepared against and standardized with a preparation of human serum albumin, obtained from the pooled plasma of several hundred normal human adults, by precipitation from an alcohol-water mixture at low temperature with controlled pH, ionic strength, and protein concentration and subsequently by crystallization with a small amount of decanol and by two recrystallizations. It might be argued that such an albumin preparation might not represent the total albumin of normal human plasma and that another albumin may have been present in normal plasma, in nephrotic plasma or in both which would not react with antibody prepared against such a crystallized albumin. Under such circumstances this albumin component could have escaped detection throughout these studies. Against this is the close agreement between values obtained for the albumin content of nephrotic sera and urine by immunochemical analysis and those obtained by the electrophoretic method. Moreover, it is extremely unlikely that two proteins, derived from the same source and with identical physico-chemical properties, would not cross-react at all when studied immunochemically (20).

SUMMARY

An immunochemical study was made of the serum albumin of 11 children with the nephrotic syndrome employing rabbit and horse antisera. The albumins of the ascitic fluid from eight of these patients and an additional patient, of the urine from six patients and of the edema fluid from two patients were studied also. In all instances, the nephrotic albumin was immunochemically indistinguishable from crystallized normal human serum albumin. The average albumin level in the serum of the nephrotic patients studied, as determined immunochemically, was 0.3 gm.%, with levels ranging from 0.09 gm.% to about 0.6 gm.%.

The serum albumin of a patient with severe chronic glomerulonephritis was also immunochemically indistinguishable from normal serum albumin. However, the serum albumin concentration in this patient was 1.57 gm.%.

ACKNOWLEDGMENT

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SPONTANEOUS FLUCTUATIONS IN GLUCOSE CONTENT OF THE HEPATIC VENOUS BLOOD IN RESTING NORMAL HUMAN BEINGS

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There is ample evidence that the liver helps the fasting animal to maintain the blood glucose concentration at normal levels by secreting glucose into the hepatic vein. After removal of the liver from experimental animals, hypoglycemia develops rapidly (1). In animals the concentration of glucose in the hepatic vein is usually higher than in the portal vein or the arteries (2). The application of the technique of venous catheterization has made it possible to show that similar relationships usually are found in the normal, unanesthetized human subject (3, 4).

It has usually been assumed that, under basal conditions, the secretion of glucose into the blood stream by the liver is a continuous process, and consequently, a fair estimate of the rate of release of glucose can be obtained by multiplying the difference between the arterial and hepatic venous glucose concentrations (A–HV) by the estimated hepatic blood flow (EHBF). The observations set forth in this paper indicate that the rate of release of glucose by the liver is highly variable, and that even the examination of a large number of A–HV pairs may fail to produce a reliable estimate of the rate of production of glucose by the liver.

METHODS

The subjects were 11 normal male volunteers convalescent from various diseases (Tables III and V). Most of the subjects were sedated with 0.5 gm. sodium amytal intramuscularly 30 minutes before the procedure and were drowsy but responsive when the samples were obtained. The intravenous catheter was inserted into the right antecubital vein and guided under direct fluoroscopic control into a right hepatic vein. Its position was adjusted until a free reflux of blood could be obtained (more than 10 ml. per minute). A Cournand needle was placed in the left brachial or femoral artery. In most subjects, a priming dose of 150 mg. bromsulfalein was immediately given intravenously, and following this a solution of bromsulfalein was infused intravenously, using an electric pump to deliver a constant quantity of the dye. At the end of the experiment, a five-minute collection of dye from the pump was analyzed for bromsulfalein and the rate of infusion calculated from this figure. The rate of infusion was between 2 and 3 mg. per minute.

A period of 30 minutes was permitted to elapse after the start of the bromsulfalein injection. During this time the patient was reassured that no further operative procedure was contemplated, and most patients went to sleep.

After 30 minutes, the catheter was cleared of saline before sampling by withdrawing 5 ml. of blood, which were discarded. Specimens were then drawn simultaneously from the arterial needle and hepatic venous catheter. Five ml. were preserved in fluoride-oxalate mixture, and analyzed for glucose. The copper tungstate filtrates (5) for glucose analysis were made within two hours after drawing the blood. The remainder of the blood was permitted to clot, and the serum analyzed for bromsulfalein. The procedure was repeated for additional samples at the time intervals indicated in the tables.

In most patients, the hepatic blood flow was estimated by the method of Bradley, Ingelfinger, Bradley and Curry (6). Splanchnic glucose balances were estimated by multiplying A–HV by EHBF.

GLUCOSE ANALYSIS

The detection of significant differences between arterial and hepatic venous glucose concentrations is limited by the accuracy of the glucose method. The error of the method, in turn, is a composite of a number of different errors. In order to estimate the accuracy of the Nelson method (7) and to assign the possible sources of error their proper importance, a series of tests was done.

It was found that the color developed by the addition of the arsenomolybdate solution at room temperature increases gradually to a plateau, which is not reached for 120 minutes. The increment is small, representing only a 5% increase; but a variation of 5% may be greater than the value of A–HV. All samples were therefore permitted to stand for 120 minutes or more before they were read in a Coleman Jr. spectrophotometer at 650 mμ.

An attempt was made to discover whether variations in the technique of protein precipitation cause any significant error in the method. Six separate protein precipitations (5) were made from a single, well-mixed sample of blood. In three, Ostwald pipettes were permitted to drain for 30 seconds after they had completely discharged their

TABLE I

Analysis of variance of the Nelson glucose method
Quadruplicate determinations in multiple protein-free filtrates prepared from the same blood specimen.

Filtrate No.	1	2*	3	4*	5	6*
mg./100 ml.	62.0	61.9	61.9	61.8	62.0	61.2
	61.5	62.1	61.2	61.8	61.8	61.5
	62.0	61.8	61.0	61.2	61.5	62.0
	61.4	61.1	61.3	61.6	62.0	62.0
Mean	61.7	61.7	61.4	61.6	61.8	61.7

* 1 ml. Ostwald pipettes drained completely and then rinsed out.

Group variance = 0.1620
Individual variance = 0.2038
F = 0.7948

Variance within filtrates is greater ($p < .01$) than between filtrates.

contents of blood, and then were blown out once. In three others, the blood was permitted to drain into distilled water in a centrifuge tube, and the pipettes were then rinsed out thrice with the distilled water. Copper sulfate and sodium tungstate were added from volumetric pipettes, and the distilled water was measured with a calibrated burette. The results, shown in Table I, were submitted to an analysis of variance (8), which shows that there is greater variation from one tube to another in the same filtrate than there is between various filtrates. It made no difference whether the pipette was blown out or rinsed out. Consequently, the pipettes were rinsed out routinely, since this proved to be more rapid. All pipettes were calibrated by weighing the quantity of distilled water discharged under standard conditions. The maximum variation among pipettes was less than 1%.

The analysis of variance proved that no advantage was gained by making multiple protein-free filtrates from the same sample. It was necessary, however, to estimate the number of replicates from each protein-free filtrate which had to be analyzed in order to afford the degree of accuracy desired. Table II shows the relationship between the number of replicates and the minimum difference detectable between two samples, with a $p = .02$. On the basis of these data, quadruplicate analyses were performed on all protein-free filtrates. This made it possible to detect a difference of 1.6 mg./100 ml. between samples in the range of 100 mg. glucose/100 ml., with a probability of 2%.

TABLE II

Effect of varying the number of replicates on the minimum detectable difference between two samples of glucose (Nelson method)
Based on data in Table I. Mean glucose concentration = 61.7 mg./100 ml.

No. of replicates	Degrees of freedom	Standard error of the difference (mg./100 ml.)	Difference for p = .02 (mg./100 ml.)	Difference: % of mean
2	2	0.44	3.1	5.0
3	4	0.36	1.4	2.3
4	6	0.31	1.0	1.6
5	8	0.28	0.8	1.3

RESULTS

A–HV pairs were drawn at 10-minute intervals in four subjects (Table III). During this time, considerable variation occurred in both arterial and hepatic venous glucose concentrations. In three of the four subjects, the hepatic venous blood contained less glucose than the arterial on at least one occasion. In these subjects, the variations in A–HV difference and in splanchnic balance were so great that the mean values were not significantly different from zero. In two instances the mean values for A–HV indicated a net *retention* of glucose by the liver, although this apparent retention was not significant when analyzed statistically.

Further insight into the rate of change of A–HV was sought by obtaining specimens at two-minute intervals in three of the subjects who had also been studied at 10-minute intervals (Table IV). The variations of A–HV were even more erratic when examined at this short time interval than when observed at 10-minute periods. The mean value for A–HV was negative in each case, indicating that the liver was releasing glucose into the systemic circulation, but the variation was so great that the mean was not significantly different from zero in any case.

These observations made it clear that the determination of isolated sets of A–HV pairs gives an unreliable picture of the overall splanchnic glucose balance. An attempt was therefore made to estimate the mean A–HV difference by taking a continuous five-minute sample. It was hoped that this would make possible a direct integration of the difference between the arterial and hepatic venous glucose concentration curves.

The method of continuous sampling was as follows: The subject was given 2.5 ml. (25 mg.) heparin solution through the catheter immediately after the arterial needle had been placed. Since the object of the experiment was simply to follow A–HV differences, no estimation of hepatic blood flow was attempted. After 20 minutes, a length of plastic tubing approximately 10 cm. in length was attached to the Cournand needle and closed off with a pinchcock. A three-way stopcock was attached to the catheter and the I.V. saline drip which serves to keep the catheter clear was disconnected. Using a 10 ml. syringe, blood was continually aspirated from the catheter at a rate of 1 ml. every five seconds. At the end of 25

TABLE III

Splanchnic glucose balance, measured at 10-minute intervals

Patient	Age, Sex	Admission diagnosis	Sedation	0 Minutes					10 Minutes					20 Minutes				
				A*	HV	EHBF	A–HV	Bal	A	HV	EHBF	A–HV	Bal	A	HV	EHBF	A–HV	Bal
J. H. B.	26 M	Pneumococcal pneumonia	Na amytal 0.5 gm. I.M.	117	90	593	+27	+160	110	128	791	−18	−142	109	104	612	+ 5	+31
E. P.	24 M	Lymphocytic meningitis	Na amytal 0.5 gm. I.M.	101	91	905†	+10	+ 91	110	112	835	− 2	− 17	100	96	995	+ 4	+40
L. W.	41 M	CNS syphilis	Na amytal 0.5 gm. I.M.	123	138	727	−15	−109	120	135	592	−15	− 89	112	128	555	−16	−89
R. T.	41 M	Tabes dorsalis	None	79	86	979	− 7	− 69	79	84	661	− 5	− 33	77	80	780	− 3	−23

Patient	Age, Sex	Admission diagnosis	Sedation	30 Minutes					40 Minutes					50 Minutes					Mean Bal	Mean A–HV	Mean EHBF
				A	HV	EHBF	A–HV	Bal	A	HV	EHBF	A–HV	Bal	A	HV	EHBF	A–HV	Bal			
J. H. B.	26 M	Pneumococcal pneumonia	Na amytal 0.5 gm. I.M.	138	114	759	+24	+182	103	101	790	+ 2	+16	93	104	757	−11	−83	*+27 ±52.4*	*+4.8 ±7.39*	672 ±37
E. P.	24 M	Lymphocytic meningitis	Na amytal 0.5 gm. I.M.	110	99	796	+11	+ 88	103	107	995	− 4	−40						*+34 ±26.7*	*+3.8 ±3.04*	905 ±52
L. W.	41 M	CNS syphilis	Na amytal 0.5 gm. I.M.	120	125	491	− 5	− 25	131	142	651	−11	−72	133	128	700	+ 5	+35	*−58 ±22.0*	*−9.5 ±3.34*	619 ±37
R. T.	41 M	Tabes dorsalis	None	73	81	780	− 8	− 62											−47 ±11.0	−5.8 ±1.12	800 ±66

* A, HV and A–HV are expressed as mg. glucose/100 ml. blood. EHBF is expressed as ml./M² body surface/minute. Bal represents the splanchnic glucose balance in mg./M²/minute. Where A–HV or Bal are negative, the splanchnic system is releasing glucose into the circulating blood. Italicized values are not significantly different from zero.

† Mean of other four determinations—BSP concentration not at equilibrium for 1st period and EHBF inaccurate.

TABLE IV

Arterio-hepatic venous glucose differences, measured at two-minute intervals

Patient	0 Minutes			2 Minutes			4 Minutes			6 Minutes			8 Minutes			10 Minutes			Mean	Standard error
	A*	HV	A-HV	A	HV	A-HV	A	HV	A-HV	A	HV	A-HV	A	HV	A-HV	A	HV	A-HV		
J. H. B.	77	105	−28	77	106	−29	98	91	+ 7	83	97	−14	131	117	+14	110	128	−18	*−11*	±7.4
E. P.	101	103	− 2	99	99	*0*	98	118	−20	94	99	− 5	103	105	− 2	110	112	− 2	*− 5*	±3.0
L. W.	126	141	−15	158	132	+26				113	134	−21	121	141	−20	120	135	−15	*− 9*	±8.8

* See footnote to Table III.

seconds, the stopcock was turned and the blood ejected from the side-arm into a fluoride-oxalate bottle. The same syringe was then used to draw the next sample in the same manner. The arterial sample was collected by permitting the blood to run freely from the plastic tube, using the pinchcock only to permit the receptacle to be changed every 30 seconds. A total of 10 pairs of specimens, representing a total of five minutes of continuous sampling, was obtained in each subject.

The individual samples were analyzed for glucose; and in addition integrated samples, representing the entire five-minute period, were made by pooling 1 ml. aliquots from all of the arterial and all of the hepatic venous specimens.

The results of the 30-second sampling are shown in Table V. The arterial glucose concentrations were subject to slight but statistically significant fluctuations. These variations, however, were small in comparison with the alterations in the glucose concentration in hepatic venous blood. In some instances, this variation still does not alter the consistently negative pattern of A–HV (J. B., J. L. T.); but in other cases periods of significant glucose retention were seen (V. H. 120″ to 180″; J. C. 210″ to 240″). The last columns of Table V compare the results of graphic integration of the A–HV difference, using a polar planimeter, with "chemical integration," using the mixed samples. For reference, the mean ± standard error of the A–HV difference are also shown. The graphic integration and the arithmetic mean of the A–HV values are in good agreement. The mixed samples, however, do not agree well with the other two. The cause of this discrepancy is not immediately apparent. It probably represents the fact that the analytic error for the mixed sample is increased by the errors of pipetting the aliquots, mixing, etc. Moreover, errors in the 10 A–HV pairs would be expected, on the basis of probability theory, to be distributed around zero, and therefore to tend to cancel out, whereas no such correction occurs in the single set of chemical determinations performed on the mixed sample. It is quite clear therefore that chemical integration by mixing is much less accurate than the determination of multiple A–HV pairs. The same is doubtless true if a single pair of samples is drawn by constant sampling for five minutes. But even when multiple pairs are analyzed, the spontaneous variation of the blood glucose concentration is so great as to cast doubt on the significance of the estimation of splanchnic balances from these data. This is illustrated in the table where the 95% limits of confidence are listed for the four patients. This column gives for each patient the range within which the mean of the A–HV differences will fall 95% of the time if the experiment is performed repeatedly, assuming that the variability of blood glucose concentration does not change. From this column it can be seen that even when 10 pairs of samples are taken, the mean value of A–HV cannot be defined with surety.

When the values for A–HV are followed, it is apparent that three of the four subjects had short periods of greatly accelerated glucose output (J. C. 30–60; V. H. 0–30, 60–90; J. L. T. 90–150). No cause for these sudden "spikes" of glucose release is apparent. In view of the well-known effects of emotion and stress on the blood glucose concentration (9), it seemed possible that these fluctuations might represent reactions to minor traumata not perceptible to the examiner—possibly even of an unconscious nature. To test this hypothesis, three subjects, two of whom had received no sedation, were subjected to a sudden, very loud noise, 120 seconds after the sampling had started. The results of this experiment are shown in the lower part of Table V. In one subject (R. W.) a well-defined "spike" occurred 120 seconds after the noise; in the other two subjects no such response

TABLE V

Arterio-hepatic venous difference, measured at 30-second intervals

Patient	Age, Sex	Admission diagnosis	Sedation	0–30 Seconds			30–60 Seconds			60–90 Seconds			90–120 Seconds			120–150 Seconds			150–180 Seconds			180–210 Seconds		
				A*	HV	A–HV	A	HV	A–HV	A	HV	A–HV	A	HV	A–HV	A	HV	A–HV	A	HV	A–HV	A	HV	A–HV
J. C.	19 M	"Smoke" intoxication	Na amytal 0.5 gm. I.M.	94	103	− 9	87	111	−24	95	100	− 5	97	96	+ *1*	104	114	−10	Clotted sample			95	103	− 8
J. B.	34 M	Pneumococcal meningitis	Na amytal 0.5 gm. I.M.	104	129	−25	96	123	−27	102	127	−25	104	122	−18	94	113	−19	99	120	−21	107	126	−19
V. H.	47 M	Paresis; epilepsy	Na amytal 0.5 gm. I.M.	126	181	−55	134	142	− 8	144	194	−50	141	167	−26	153	139	+14	155	151	+ 4	123	147	−24
J. L. T.	37 M	Right lower lobe lung abscess	Na amytal 0.5 gm. I.M.	104	117	−13	107	118	−11	107	122	−15	101	132	−31	105	139	−34	108	116	− 8	116	123	− 7
																			Loud noise at 120 seconds					
R. W.	53 M	Exfoliative dermatitis	None	126	109	+17	139	133	+ 6	124	130	− 6	130	130	*0*	133	135	− 2	121	116	+ 5	125	126	− *1*
C. D.	47 M	Epilepsy? psychoneurosis?	None	166	108	+58	123	163	−40	142	146	− 4	133	173	−40	178	137	+41	150	165	−15	135	132	+ 3
J. T.	49 M	Chronic alcoholism; liver function normal	Na amytal	111	103	+ 8	128	123	+ 5	89	134	−45	92	138	−46	126	117	+ 9	145	153	− 8	137	136	+ *1*

Patient	Age, Sex	Admission diagnosis	Sedation	210–240 Seconds			240–270 Seconds			270–300 Seconds			Mean A–HV	95% confidence limits	Mixed sample "Chemical Integration"			Integrated with planimeter
				A	HV	A–HV	A	HV	A–HV	A	HV	A–HV			A	HV	A–HV	
J. C.	19 M	"Smoke" intoxication	Na amytal 0.5 gm. I.M.	104	101	+ 3	100	99	+ *1*	100	101	− *1*	− *5.8 ±2.78*	−0.6 to −12.2	105	104	+ 1	− 5.3
J. B.	34 M	Pneumococcal meningitis	Na amytal 0.5 gm. I.M.	103	124	−21	97	104	− 7	114	121	− 7	−18.9 ±2.19	−14 to −23.9	109	118	− 9	−16
V. H.	47 M	Paresis; epilepsy	Na amytal 0.5 gm. I.M.	128	152	−24	139	135	+ 4	133	132	+ *1*	−16.4 ±7.42	−0.6 to −33.2	129	139	−10	−14
J. L. T.	37 M	Right lower lobe lung abscess	Na amytal 0.5 gm. I.M.	Clotted sample			Clotted sample			124	128	− 4	−15.4 ±3.94	−6.1 to −24.7				
R. W.	53 M	Exfoliative dermatitis	None	124	158	−34	116	125	− 9	98	114	−16						
C. D.	47 M	Epilepsy? psychoneurosis?	None	174	147	+27	164	167	− 3	135	107	+28						
J. T.	49 M	Chronic alcoholism; liver function normal	Na amytal	145	77	+68	120	133	−13	123	111	+12						

* See footnote to Table III.

occurred. It is noteworthy that C. D. had widely fluctuating A–HV values during the entire procedure, in spite of the fact that he did not appear anxious at any time.

DISCUSSION

This study has evaluated the variations in glucose production by the splanchnic system of human beings under experimental circumstances which protect them, as far as possible, from disturbing influences. The subjects were fasting, resting in a quiet environment and (except in two special cases) sedated to the point of moderate drowsiness. All manipulations were performed under local anesthesia, and pain stimuli were practically absent. Psychic stimulation arising from the strangeness of the environment, resentment at missing breakfast, etc., were undoubtedly present. It is impossible to evaluate the role which these factors played in disturbing the experimental subjects, but there can be no doubt that emotional influences may have been very important. It seems probable, however, that their effect was the least which could be achieved in this type of study.

In spite of the favorable environment and relative lack of overt stimulation, the observations reported above show clearly that rapid, unpredictable fluctuations occur in the splanchnic glucose balance, and that these variations are so large as to interfere seriously with attempts to estimate the mean balance over any protracted period of time.

The changes in splanchnic glucose balance are probably the results of at least three factors. The first and probably least important is experimental error. The data pertaining to the accuracy of the glucose determination indicate that only a very small portion of the variation could be a result of errors in this estimation.

A second cause of variation is a result of changes in the splanchnic blood flow. The fluctuations in the hepatic blood flow as estimated by the methods available are considerable (coefficient of variation 12.4%) but since one cannot obtain true duplicate determinations, it is impossible to estimate how much of the variation is due to errors of analysis and how much to actual physiological alterations in the blood flow. In any case, variations of splanchnic balance of the order of 15 or 20% may be accounted for by fluctuations of splanchnic blood flow.

The studies of the A–HV difference, however, show that even this source of variation is relatively minor in comparison with the rapid changes in splanchnic A–HV glucose differences. It seems probable that most of the change observed in the value of A–HV is due to actual variations in the rate of production of glucose by the liver and in the rate of utilization of glucose by the gut.

It has been shown that variations in the arterial glucose concentration can cause variations in the splanchnic glucose balance (3). In the present study, the arterial glucose concentration was not manipulated experimentally and remained fairly constant. In no case did it reach levels of definite hyper- or hypoglycemia. It seems unlikely therefore that the rapid variations of A–HV glucose difference were a response to alterations of the arterial glucose concentration.

Hinkle (9) has shown that rapid alterations in the venous glucose concentration can be produced by psychic stimulation. The importance of this factor was not investigated directly in the present study, except in three subjects who were startled suddenly by a loud noise. This type of stimulus is admittedly a naive one, since its psychological significance will vary from one subject to another. Moreover, the effect of such an extraneous stimulus might be simply to distract the subject from his more significant reveries. The advantage of the single stimulus, however, in making possible accurate timing of the response, more than outweighed the psychiatric disadvantages in the present study. In one of these subjects, a "spike" of glucose output occurred after about the time interval which might have been expected if it resulted from the release of epinephrine. The other two subjects showed no such response. These observations indicate, at best, that "startle" reactions might be able to produce fluctuations comparable to those seen spontaneously. There is no direct evidence, however, that this type of reaction is actually involved in the production of the fluctuating A–HV difference.

The variability of the splanchnic glucose balance under rigidly controlled "basal" conditions raises grave doubts whether these observations have any relationship to the normal splanchnic glu-

cose balance under the vicissitudes of daily life. The momentary fluctuations observed under these specially protected conditions are probably negligible in comparison with the variations induced by ordinary living. It is therefore questionable whether the estimations obtained by liver-catheterization techniques have any validity as representations of the daily 24-hour balances. Although these estimations have, in the past, been used as the basis for calculating the metabolic mixture and glucose utilization (3, 4), no confidence can be given to such calculations because (*1*) the estimates of A–HV have been based upon too few observations, and (*2*) even with many more observations, the value for A–HV (and therefore for splanchnic balance) would have meaning only with regard to the moments during which the estimation was being made.

SUMMARY AND CONCLUSIONS

The arterio-hepatic venous glucose difference and hepatic blood flow have been estimated at intervals varying from 30 seconds to 10 minutes in 11 normal male human subjects under basal conditions. There is a very great spontaneous (and probably physiological) variation in the splanchnic glucose balance. In view of this unpredictable fluctuation, it is doubtful that estimates of splanchnic glucose balance are valid for any periods other than the actual time of sampling. Moreover, unless constant sampling techniques are used, the estimate of the splanchnic balance is valid only for the instant during which blood was actually being withdrawn.

In view of these findings, it seems unwise to use data obtained by the liver-catheter technique to estimate overall metabolic balances or rates of glucose utilization.

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AGGLUTINATION OF SENSITIZED SHEEP ERYTHROCYTES AND COLLODION PARTICLES BY TUBERCULOUS AND NORMAL SERA [1]

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The agglutination of sensitized erythrocytes by antisera directed against the sensitizing substance was first applied to a study of tuberculous sera by Middlebrook and Dubos (1). Its dependability for diagnosis of the disease was immediately explored by a number of investigators. Scott and Smith (2) reported remarkable specificity of the reaction using a special preparation of Old Tuberculin concentrated to four times standard strength, as did Rothbard, Dooneief and Hite (3). The latter authors state that the test is a valuable diagnostic tool, titers of 1:8 and over being indicative of tuberculous activity. Others (4–6), including Smith and Scott (4) in a second more extensive study, found less correlation between hemagglutination titer and either the presence or the activity of the tuberculous process. The present report is a further attempt to evaluate the usefulness of the hemagglutination test in the detection of specific antibody for the tubercle bacillus or its fractions and to compare results with those obtained using collodion particles instead of sheep erythrocytes as carriers for antigens (7–9).

METHODS

Antigens. Sheep erythrocytes were sensitized with either Old Tuberculin, four times standard strength (2),[2] or with an aqueous extract of strain H37 Rv tubercle bacilli obtained by distilled water refluxing of dried, washed bacilli which had been grown on glycerol beef extract broth and briefly extracted with acetone. The main part of the bacillary debris in the aqueous extract was removed by centrifugation. The remaining opalescent solution, which gave positive color reactions with both Molisch's and Millon's reagents, was used as the antigen. Antigen prepared in this manner was originally used in the complement fixation test for tuberculosis (10). O.T. in our hands was not satisfactory for sensitizing collodion particles since it caused non-specific agglutination of particles; hence, only the aqueous extract was used in collodion particle agglutination tests. Polysaccharide from the Olson strain of tubercle bacillus [3] was found useful in the agglutination-inhibition reaction (1), but neither erythrocytes nor collodion particles treated with this polysaccharide in the concentrations used were agglutinated by human tuberculous sera.

Sera. Blood serum was obtained from (*a*) tuberculous human patients and (*b*) non-tuberculous individuals. The non-tuberculous group consisted of 21 apparently healthy, supposedly normal adults plus seven children who had visited the out-patient department of The Childrens Memorial Hospital for various reasons. Mantoux reactions made two months or more prior to obtaining blood samples were negative in 17 and positive in 11 of these individuals. Tests were performed either within a few days of drawing the blood or else on sera which had been kept frozen. Sera were inactivated at 56° C. for 30 minutes immediately before use. Two-fold serial dilutions beginning with 1:4 were made in either phosphate buffered or unbuffered physiological saline for hemagglutination tests and in 1:100 normal rabbit serum in 1.1% NaCl (11) for collodion particle agglutination tests. Controls consisted of sensitized carrier plus diluent, 1:4 dilution of serum plus unsensitized carrier, and unsensitized carrier plus diluent. Known agglutinating and non-agglutinating human serum controls were set up each time tests were run.

Hemagglutination test technique. Sheep erythrocytes were collected in modified Alsever's solution (10) and washed three times with either physiological saline or buffered saline solution. Adsorption of aqueous extract was in the proportion of 0.1 ml. packed washed red cells to 2 ml. of isotonic antigen. In the case of four-times concentrated O.T., 1 volume of washed, packed sheep cells was adsorbed either with 48 volumes of 1:11 dilution of antigen as described in the report of the Laboratory Committee of the V.A. Central Office Streptomycin Committee (June 2, 1950), or with 60 volumes of 1:15 O.T. as described by Scott and Smith (2). Two-fold

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[3] Supplied through the kindness of Dr. W. J. Nungester, Department of Bacteriology, University of Michigan, Ann Arbor.

serial dilutions of sera previously absorbed with washed unsensitized sheep cells to remove heterophile antibody were set up in 0.4 ml. amounts to which an equal volume of sensitized cells was added. Sensitized cells were first used in 0.5% suspension, later changed to 0.2% to facilitate reading of tests. Readings were made after two hours' incubation in a 37° C. water bath followed by overnight incubation at room temperature. Tubes were read, after noting pattern formed by settling of the cells, following gentle resuspension and the amount of agglutination estimated.

An analysis of hemagglutination test results indicated that in our hands it was not a satisfactory diagnostic test. The hemolytic modification (12) was then used with a number of sera to determine whether or not it would detect tuberculous antibody with greater specificity.

Technique for the hemolytic modification of the hemagglutination test. The hemolytic modification was performed as described by Middlebrook (12). An excess of complement which had been previously absorbed twice with unsensitized sheep erythrocytes at refrigerator temperature was added to each tube of the regular hemagglutination test. Appropriate controls were included. Tests were read for resulting hemolysis after one hour's incubation in a 37° C. water bath.

Collodion particle agglutination technique. Equal portions of antigen and concentrated collodion particles prepared by Cavelti's method (11) were mixed and allowed to stand for 30 to 40 minutes at room temperature, with occasional shaking, and then stored overnight in the refrigerator. Before use, they were diluted with 1 : 100 normal rabbit serum in 1.1% NaCl solution, centrifuged lightly (1,400 r.p.m.) in an International No. 2 SB centrifuge for five minutes; the supernate was then decanted and centrifuged similarly. Centrifuging in this manner was repeated until the supernate was clear (four to six times). Sediments were resuspended, pooled, and diluted to the density of a No. 2 McFarland standard with 1 : 100 normal rabbit serum in 1.1% NaCl. Normal rabbit serum in 1.1% NaCl was used following Cavelti's suggestion that it helped eliminate non-specific agglutination of particles by human sera (11). Equal portions of unsensitized particles and three-times distilled water (instead of antigen) were similarly prepared for serum controls for non-specific agglutination.

Equal portions of sensitized collodion particles and serum dilutions were incubated at room temperature for one to two hours. Following centrifugation at low speed (1,400 r.p.m.) for three minutes, preliminary readings were made against a dark background, using a bright light source and a concave mirror for magnification. Final readings were made in a similar manner after tests had stood overnight in the refrigerator.

RESULTS

Hemagglutination and collodion particle agglutination. Results are summarized in Table I. It will be noted that the percentage of high titers (1 : 8 and over [3] or 1 : 16 and over [12]) in the tuberculous group was higher than in the non-tuberculous group by both methods and with both antigens. Differences between non-tuberculous and tuberculous sera were greater if 1 : 16 were accepted as the "significant" titer. The range of titers of tuberculous sera was one two-fold dilution higher than the range with non-tuberculous sera; although this difference was slight, it was consistent with both sensitizing substances and both carriers. Significant differences (*i.e.*, at least a four-fold dilution) in average titers (modal and median) of tuberculous and non-tuberculous sera occurred with aqueous extract-sensitized erythrocytes; there was less difference in average titers of the two groups of sera using O.T.-sensitized red cells and aqueous extract-sensitized collodion particles. The high percentage of non-tuberculous sera which agglutinated both types of sensitized carriers in dilutions of 1 : 16 and over (28% to 39%), although lower than the percentage of tuberculous sera (54% to 74%), appeared to pre-

TABLE I

Hemagglutination and collodion particle agglutination titers of non-TB and TB sera compared

	Hemagglutination Ag:* Aqueous extract		Hemagglutination Ag:* O. T.		Collodion particles Ag:* Aqueous extract	
	Non-TB† sera	TB sera	Non-TB† sera	TB sera	Non-TB† sera	TB sera
No. of individuals	28	43	21	46	28	43
Median titer reciprocal	4	32	8	16	8	16
Modal titer reciprocal	4	32	0 or 8	16	8	16
Range of titers	0 to 64	0 to 128	0 to 32	0 to 64	0 to 64	0 to 128
Titers 1:8 and over	15 or 53%	35 or 81%	12 or 57%	34 or 73%	18 or 64%	34 or 79%
Titers 1:16 and over	11 or 39%	32 or 74%	6 or 28%	25 or 54%	10 or 35%	28 or 65%

* Ag: = Antigen.
† Non-TB = 17 Mantoux neg. and 11 Mantoux pos. (see Table II).

TABLE II

Non-TB sera: Hemagglutination titers of Mantoux negative and Mantoux positive individuals compared

	Hemagglutination Ag:* Aqueous extract		Hemagglutination Ag:* O. T.		Collodion particles Ag:* Aqueous extract	
	Mantoux neg.†	Mantoux +	Mantoux neg.†	Mantoux +	Mantoux neg.†	Mantoux +
No. of individuals	17	11	11	10	18	10
Median titer reciprocal	4	16	8	4	8	4 to 8
Modal titer reciprocal	4	16	8	0	8	4
Range of titers	0 to 64	0 to 64	0 to 32	0 to 32	0 to 32	0 to 64
Titers 1:8 and over	8 or 47%	7 or 63%	8 or 72%	4 or 40%	13 or 72%	5 or 50%
Titers 1:16 and over	5 or 29.4%	6 or 54%	4 or 36%	2 or 20%	6 or 33%	4 or 40%

* Ag: = Antigen.
† Negative to at least second strength PPD.

clude the use of tests as employed in this work for diagnostic purposes.

Even though correlation of antibody titer and skin reactivity to tuberculin have not previously been observed, it is conceivable that serum of individuals having positive tuberculin skin tests might, as a result of previous infection, contain specific circulating antibody. Lack of correlation between skin reactivity to tuberculin and agglutinating titer, however, is evident in Table II, in which the nontuberculous sera were separated into two groups: those from positive and those from negative reactors (tested with at least 0.005 mg. PPD). Only with aqueous extract-sensitized erythrocytes was the percentage of "significant" titers (1:16 and over) (12) somewhat lower in the Mantoux negative than the Mantoux positive group. The lowest per cent of titers of 1:16 and over occurred with O.T.-adsorbed erythrocytes (Mantoux positive group); however, it will be noted in Table I that the lowest percentage of high-titered tuberculous sera also occurred with O.T.-sensitized erythrocytes. There is little difference in average titers or range of titers between the two groups of nontuberculous sera.

Further evidence of failure of correlation of skin reactivity to tuberculin and titer of sensitized particle agglutination was obtained in the following manner. Repeated intracutaneous tuberculin tests were made on five normal, supposedly healthy individuals of the tuberculin negative group after drawing blood for agglutination tests. Successive injections of 1:10,000, 1:1000, 1:100 and 1:10 O.T. were made; as soon as tests with one dilution were read as negative, the next strongest concentration of O.T. was injected.[4] None of the five reacted to 10 mg. O.T. Sera taken before tuberculin tests had the following titers: with O.T.-sensitized red cells, 0, 1:4, 1:8, 1:8, and 1:16; with aqueous extract-sensitized red cells, 0, 1:4, 1:8, 1:16 and 1:32; sensitized collodion particles were agglutinated by dilutions of 1:4, 1:8, 1:16 and 1:32 of these five sera.

Blood serum was again obtained from these five individuals within four to seven days after the last tuberculin injection to determine whether or not repeated injections of tuberculin (four injections in two weeks' time) had resulted in increased titers. Dilutions of serum which agglutinated both O.T.-adsorbed erythrocytes and aqueous extract-adsorbed collodion particles were the same for each individual as before the repeated injections of tuberculin.

Inasmuch as negative skin reactions in children constitute more conclusive evidence than in adults of the absence of tuberculous processes, blood serum was obtained from eight patients under 12 years of age from The Childrens Memorial Hospital. Mantoux tests of seven were negative. Serum from one of the seven agglutinated aqueous extract-adsorbed erythrocytes in a dilution of 1:32. Two sera agglutinated sensitized collodion particles in dilutions of 1:16. Unfortunately the concentrated O.T. was not available at that time.

Titer and disease activity. Results with tuberculous sera were studied for possible correlation of titer with activity of the disease, graphically presented in Figure 1. It is evident that most of the

[4] We are indebted to Dr. Jennings Fershing for the tuberculin injections.

sera from patients with moderate or severe symptoms (3 or 4 activity) had high hemagglutination titers with both antigens and all of these sera agglutinated collodion particles in high dilution. Correlation of less disease activity with low titers is poor, however. Most sera from cases with little disease activity had low titers, but serum from one quiescent case agglutinated both bacillary extract-sensitized red cells and collodion particles in a dilution of 1 : 32 and O.T.-sensitized red cells in a dilution of 1 : 8. Sera from other quiescent cases agglutinated O.T.-sensitized red cells in dilutions as high as 1 : 32. Titers of sera from cases whose activity was classified as 1 (no symptoms but active) had titers ranging from 0 to 1 : 64 with bacillary extract-sensitized cells, from 0 to 1 : 32 with O.T.-sensitized cells and from 0 to 1 : 32 using sensitized collodion particles. Thus the range of titers of sera from active cases with no symptoms was wide.

Collodion particles and red cells sensitized with the aqueous bacillary extract were not always agglutinated by the same dilution of the same serum. Differences in titer of two or more doubled dilutions were noted with approximately 30% of sera tested. Bacillary extract-sensitized cells were in general agglutinated by higher dilutions of serum than were O.T.-sensitized cells.

Hemolytic modification of the hemagglutination test. The apparent lack of specificity of the hemagglutination reaction as herein employed led us to explore Middlebrook's hemolytic modification of the test (12). Both tests were run simultaneously on identical blood samples using the same lot of

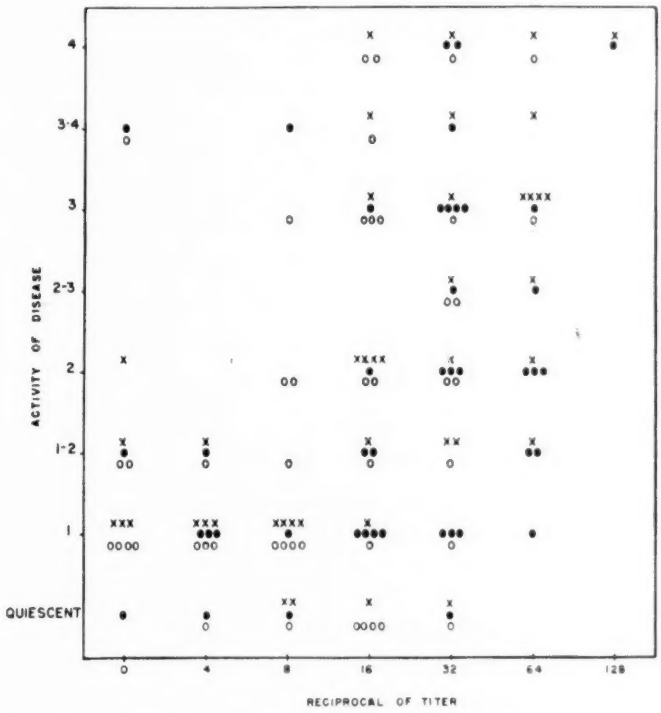

FIG. 1. TITERS OF TUBERCULOUS SERA VERSUS ACTIVITY OF DISEASE
● Hemagglutination: aqueous extract-sensitized sheep cells
○ Hemagglutination: O.T.-sensitized sheep cells
× Collodion particles: aqueous extract-sensitized

TABLE III

Hemagglutination and hemolytic modification titers of non-tuberculous sera

Antigen: Lederle's four-times concentrated O.T.

	Hemolytic modification		Hemagglutination	
	Non-TB sera	TB sera	Non-TB sera	TB sera
No. of individuals	13	21	13	21
Median titer reciprocal	0	128	8	16
Modal titer reciprocal	0	256	8, 16, 32*	16, 32†
Range of titers	0 to 32	0 to 256	0 to 32	0 to 64
Titers 1 : 8 and over	4 or 30%	18 or 85.7%	9 or 69%	19 or 90%
Titers 1 : 16 and over	3 or 23%	18 or 85.7%	6 or 46%	18 or 85%

* Three sera each.
† Eight sera each.

cells sensitized with Lederle's four-times concentrated O.T. Results are presented in Table III. It is evident that titers with the two types of tests do not correspond. In the non-tuberculous group (both tuberculin positive and tuberculin negative individuals), the distribution of hemagglutination titers is quite uniform in the range of 0 to 1 : 32, but distribution of the hemolytic titers tends to be weighted toward low values, which would indicate some improvement in specificity. The percentage of non-tuberculous sera with "significantly" high titers by the hemolytic modification, however, was still too high for the test to be of diagnostic value. The percentage of "significant" hemagglutination titers of both non-tuberculous and tuberculous groups was of the same order as in the first group (Table I), although somewhat higher in this comparatively short series of cases. Hemolytic titers of sera from the tuberculous group were in general higher than those of the hemagglutination test. The tuberculous group was chosen to contain patients with varying stages of disease activity. Symptoms of patients exhibiting high titers were as likely to be quiescent as severe; thus as with the hemagglutination test, no correlation was observed between the degree of activity of the disease and hemolytic titer.

DISCUSSION

Middlebrook (13) repeatedly speaks of the specificity of the hemagglutination reaction if the proper antigen is used for sensitizing red cells. In the hands of Scott and Smith (2) and Rothbard, Dooneief and Hite (3), Lederle's four-times concentrated O.T. appeared to constitute such an antigen. In later work with a larger number of sera, however, Smith and Scott (4) modified their views considerably, stating that the possibility of "biologically false positive or false negative reactions" had not been eliminated. The latter authors also found that sera from some tuberculin negative individuals agglutinated O.T.-sensitized sheep cells. They concluded after this study that the hemagglutination test does not determine the clinical activity of a tuberculous lesion. Results of the test as recorded in the present report support their later view.

Repeated intracutaneous injection of tuberculin in five Mantoux negative reactors failed to raise the agglutinating titer of sera for O.T-treated sheep red blood cells. Failure of correlation of skin reactivity to tuberculin and presence of agglutinins in non-tuberculous individuals is illustrated in Table II. Correlation of skin response to tuberculin and serological titer of antibody has, of course, never been previously observed (14).

Lack of correlation of results of hemagglutination and collodion particle agglutination tests with approximately 30% of sera tested, using the same antigen for sensitizing particles as herein reported, indicates that the two carriers were adsorbing different substances from the complex bacillary extract. Figure 1 suggests that the material adsorbed by collodion particles might have had greater specificity in detecting moderate or severe activity of the tuberculous process than did material adsorbed on sheep erythrocytes. The surface of a collodion particle is chemically more homogeneous than that of a red blood cell. It might therefore be expected that material adsorbed from a complex mixture would be more uniform in nature. However, high collodion particle agglutination titers of sera from quiescent cases and from cases with few symptoms, as well as with sera from healthy individuals, dampens enthusiasm for the test, at least with this antigen, for diagnostic purposes. Results with the hemolytic modification of the hemagglutination test perhaps indicate a trend toward greater specificity but fall far short of results desired in a diagnostic test. Middlebrook (12) has stressed the importance of a suitable antigen for specificity of the hemagglutination test and its hemolytic modification. It would appear that the antigens used in the work herein reported are not sufficiently specific for diagnostic purposes.

Differences between hemagglutination and hemolytic titers of the same sera were also noted by

Middlebrook (12), who discussed, as a reason for failure of correlation, the possibility that the antibodies involved in the two reactions were different in certain sera. Precise 50% hemolysis end-points were not employed for complement dosage in either Middlebrook's work, as he points out, or in the present studies. It is possible that more rigorous control of the dose of complement and other variables might yield somewhat different and possibly more satisfactory results.

Lack of a suitable antigen may be mainly responsible for failure of these tests to differentiate between tuberculous and non-tuberculous sera. Irregularities of titers with tuberculous sera could, for example, be explained by infection with different types of human tubercle bacilli containing different antigens, analogous to types of pneumococci. Keogh, North and Warburton (15) have indicated the polysaccharide nature of antigens from organisms such as *H. influenzae* and certain other bacteria which sensitize red cells for agglutination with specific antisera. Furthermore, Middlebrook's first observation of the hemagglutination reaction with tuberculous sera was obtained with red cells sensitized with a polysaccharide fraction of the tubercle bacillus. Although specific carbohydrates conferring type specificity on tubercle bacilli have not yet been recovered, they may nevertheless exist. It is known that immunization of guinea pigs with one strain confers a degree of immunity for other strains; this fact does not argue against the possible occurrence of type specific carbohydrates, however, inasmuch as the immunizing fraction of the tubercle bacillus is likewise unidentified.

In spite of the questionable feasibility for diagnosis of these reactions as presently employed, the fact that high titered sera (1 : 16 and over) were obtained from tuberculous individuals in significantly higher percentages than from non-tuberculous individuals should probably not be ignored. The natural inference is that the proper antigen has not been used. Two other possible explanations for irregularities present themselves. In the first place, assuming that positive tests detect specific antibody, failure of these as well as other serological tests in the diagnosis of tuberculosis could be accounted for by either qualitative or quantitative peculiarities of antibody for the tubercle bacillus or by its irregular occurrence in a free state in the fluid portion of the blood.

It is also possible that, instead of measuring specific antibody for the tubercle bacillus, these tests reflect rather a general non-specific host response to an irritant. Two abnormal constituents of serum, presumably results of tissue destructive processes, have already been demonstrated, that is, (*a*) the pneumococcus C-reactive protein found in sera from persons in the acute stages of a variety of diseases (16–20) and (*b*) a substance associated with the alpha$_2$ globulin of blood serum which appears to increase in tuberculosis, cancer and other diseases involving tissue destruction (21–23). McCarty points out (17) that C-reactive protein may be only one of several new substances, unrecognized simply for lack of suitable reagents for testing for their presence, to appear in the blood in the course of pathological processes. Serological results herein reported might reflect the occurrence of such a substance in blood serum rather than specific antibody.

SUMMARY AND CONCLUSIONS

Hemagglutination tests using sheep erythrocytes adsorbed with either four-times concentrated O.T. or an aqueous extract of tubercle bacilli, and collodion particle agglutination tests using the latter antigen, were performed on groups of non-tuberculous and tuberculous sera. Using O.T.-sensitized erythrocytes, titers of 1 : 16 and higher were obtained with 28% of 21 non-tuberculous sera and with 54% of 46 tuberculous sera. Using aqueous extract-sensitized red cells, supposedly significant titers were obtained with 39% of 28 non-tuberculous sera and 74% of 43 tuberculous sera and using aqueous extract-sensitized collodion particles, with 35% of the 28 non-tuberculous sera and 65% of the 43 tuberculous sera. There was no correlation between presence or absence of agglutinins in non-tuberculous sera and skin-reactivity to tuberculin.

The hemolytic modification of the hemagglutination test also appeared to be unsatisfactory for diagnosis using four-times concentrated O.T. as antigen. Twenty-three per cent of 13 non-tuberculous sera and 85.7% of tuberculous sera had titers considered significant by other observers.

Moderate and severe symptoms of tuberculosis usually were correlated with high collodion particle agglutination test titers; the correlation of little or no disease activity with low titers was less

consistent. Correlation of disease activity with hemagglutination titers (either antigen) was even less constant than with collodion particles.

Repeated intracutaneous tests of five tuberculin negative supposedly healthy individuals with increasing concentrations of O.T. failed to raise either hemagglutination (O.T.) or collodion particle agglutination (aqueous extract) titers.

Agglutination of aqueous extract-sensitized erythrocytes and collodion particles by the same sera failed to yield the same end point with approximately 30% of the sera tested, indicating adsorption of different fractions of this complex antigen by the two kinds of carriers. Hemagglutination and hemolytic titers, using O.T.-sensitized red cells also differed.

In our hands, none of these tests was sufficiently specific with the antigens employed to be useful in the diagnosis of tuberculosis. The occurrence of high titers in a far higher percentage of tuberculous than non-tuberculous sera, however, is perhaps too significant to be ignored. Whether observed irregularities are due to the use of unsuitable antigens, or to peculiarities of antibody for the tubercle bacillus, or to non-specific constituents of serum as yet unidentified, remains to be determined.

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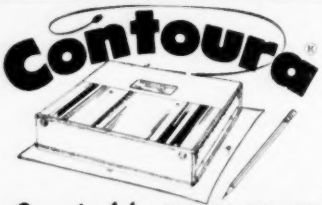

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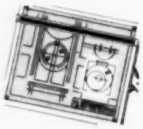

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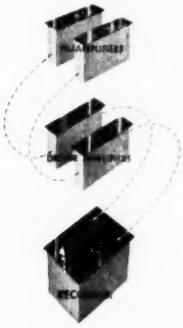

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